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Acetogen and acetogenesis for biological syngas valorization

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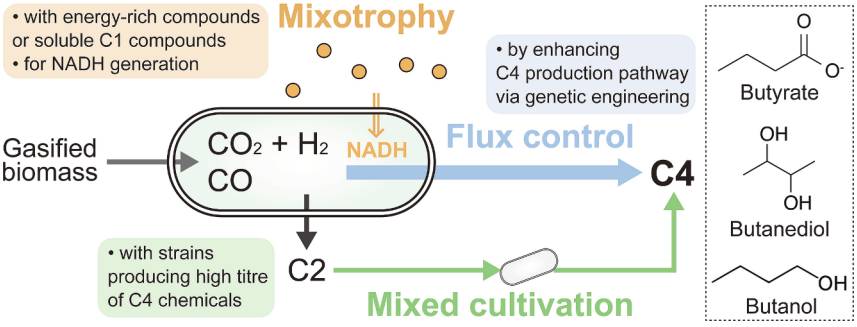
HIGHLIGHTS

- (Homo)acetogens is promising biocatalyst for syngas valorization.
- Biocatalytic syngas valorization can be applied with present infrastructure.
- Securing of reducing equivalents is important to produce valorized chemicals.
- Carbon flux and cultivation control are key to achieve C4 selectivity.

GRAPHICAL ABSTRACT



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ABSTRACT

The bioconversion of syngas using (homo)acetogens as biocatalysts shows promise as a viable option due to its higher selectivity and milder reaction conditions compared to thermochemical conversion. The current bioconversion process operates primarily to produce C2 chemicals (e.g., acetate and ethanol) with sufficient technology readiness levels (TRLs) in process engineering (as midstream) and product purification (as downstream). However, the economic feasibility of this process could be improved with greater biocatalytic options in the upstream phase. This review focuses on the Wood-Ljungdahl pathway (WLP) which is a biological syngas-utilization pathway, redox balance and ATP generation, suggesting that the use of a specific biocatalysts including *Eubacterium limosum* could be advantageous in syngas valorization. A pertinent strategy to mainly produce chemicals with a high degree of reduction is also provided with examples of flux control, mixed cultivation and mixotrophy. Finally, this article presents future direction of industrial utilization of syngas fermentation.

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1. Syngas

1.1. Biofuels and feedstock resources

Energy has played an essential role in human history and as the population of the world continues to grow, the demand for energy has risen steadily, driven by higher living standards. While fossil fuels currently serve as the primary energy source, limitations such as price fluctuations and environmental pollution caused by greenhouse gas emissions, especially CO_2, have necessitated the development of alternative energy sources (Venkata Mohan et al., 2008). The issue has led to the exploration of alternative energy sources and associated technology development (Chen et al., 2022). The Paris Agreement of 2015 recognizes this need and encourages a shift towards alternative energy, promoting promising technologies (He et al., 2020; Luderer et al., 2019). As one of the alternative energy resources, biofuels offers a type of sustainable, carbon–neutral energy source that is ubiquitously abundant and renewable (Fargione et al., 2008; Alawan et al., 2019).

Earliest version (first-generation) of biofuels was derived from edible monomeric and polymeric sugar compounds (e.g., sugarcane, sugar beet, corn and wheat), which are costly to produce and inefficient in terms of energy and resource utilization (Aron et al., 2020). In particular, utilizing edible biomass for energy and biofuels raises uncomfortable concerns about competition with food crops and the requirement of large amounts of fertilizer and water, making it an unfavorable option (Hayes et al., 2015). Second-generation biofuels made from lignocellulosic biomass are known as more sustainable and less conflicting alternatives (Aron et al., 2020; Bhatia et al., 2017). However, this feedstock has inherent characteristics that pose barriers to its development, including nitrate emissions, low conversion efficiency and deforestation (Hayes et al., 2015; Sheldon, 2018). Although using agricultural by-products to prevent deforestation can be helpful, it can only satisfy a limited portion the increased demand for biofuels (Naik et al., 2010). More recently, some marine microorganisms and algal biomass have gained attention as potential feedstocks for third-generation biofuels (Aron et al., 2020; Gambelli et al., 2017; Leong et al., 2018). Among these, microalgae are particularly promising due to their rapid growth rate, product diversity and CO_2 capture capability (Chew et al., 2018, 2017; Khoo et al., 2020, 2019; Pierobon et al., 2018). However, algae incur high cultivation costs and the resulting biofuels are unstable due to their high volatility (Shah et al., 2018). Recently, researchers looked at biofuels produced through biomass conversion processes either directly or indirectly (e.g., pyrolysis and gasification) because of that these biomass conversions might have less inherent difficulties and challenges, as mentioned earlier, in biofuel production. Synthesis gas (syngas), a type of feedstock for fourth-generation biofuels, is generated from the gasification of residual biomass and organic waste (Kanwal et al., 2022). Syngas has possibility as an energy source by itself. The feedstock could be also valorized into other chemicals. Therefore, syngas classified as the feedstock for desired biomass conversion process (Abubackar et al., 2011).

1.2. Syngas production

Syngas is typically composed of CO, CO_2 and H_2, but the proportion of these components varies based on the feedstock and production process used (Daniell et al., 2012). Thus, selecting an appropriate feedstock as the first step is crucial to designing a syngas production process. However, certain properties of biomass (e.g., low carbon content, high moisture content and heterogeneity) result in cost-intensive process configurations, despite the increasing attention in syngas production by gasification. For instance, the high ash content of rapidly growing biomass poses a particular challenge, as it may cause corrosion issues in thermochemical processing (Daniell et al., 2012). Moreover, some biomass-derived syngas contains undesired residual by-products (e.g., nitric oxide, H_2S and tar), which hinder downstream syngas conversion due to their poisoning effect on the catalyst. Unfortunately, due to the aforementioned constraints, natural gas still remains the primary feedstock for syngas production instead of biomass-derived feedstocks (Rahbari et al., 2018). In addition, technological difficulties in ratio variations of major elements (i.e., C, H and O) and impurities in gasified products degrade processing efficiency in syngas conversion stage (Rahbari et al., 2018).

1.3. Syngas conversion technology

Syngas can be converted into various chemicals and fuels via thermochemical processes such as the Fischer-Tropsch (FT) process (Munasinghe and Khanal, 2010; Schulz, 1999; Sun et al., 2019). Traditionally, the FT process has been a major process for syngas conversion into value-added chemicals. However, the efficiency of the FT process could be decreased due to inhibition by contaminants such as sulfur compounds and ammonia that are generally present in syngas (Christensen et al., 2011). Additionally, this process requires a specific syngas composition, indicating that additional purification steps are required for syngas conversion via the FT process. Moreover, the FT process requires high temperature (150-300℃), which can increase the process cost (Daniell et al., 2012). In contrast, syngas-utilizing microorganisms exhibit an efficiency of more than 57%, due to the properties of microbial reactions (Bosmans et al., 2013). Syngas is a potential source of electrons (H_2 and CO) and carbon (CO_2 and CO), which can be upcycled through biological reactions (Munasinghe and Khanal, 2010; Bengelsdorf and Dürre, 2017). Biocatalysts mainly utilize H_2:CO_2. However, they can also produce biofuels using CO as electron source, even in the absence of H_2. Additionally, biological syngas fermentation is less affected by contaminants than the thermochemical processes and does not require high temperature and pressure. Therefore, biological conversion by microorganisms via syngas fermentation represents a promising technology to fit the ideal concept of advanced utilization (Kucek et al., 2016; Marcellin et al., 2016; Richter et al., 2016).

1.4. Biological conversion

The efficiency of syngas fermentation is determined by three key processes: upstream, midstream and downstream (Table 1). The upstream process involves strain performance assessments, including strain isolation, strain development and practical use. The midstream process focuses on optimizing operating conditions at laboratory scale, such as mass transfer rate from gas to liquid, medium composition and other operating conditions (e.g., reactor, gas delivery system and pH). The downstream process reflects the optimized process at the industrial scale. It involves optimizing the fermentation process for commercialization by purifying and recovering the target product(s) and evaluating its economic feasibility. Various studies have optimized and developed operating systems for syngas fermentation in the midstream step. Purification of acids or alcohols, the main products of syngas fermentation, is also required. The technology for C2 chemical (i.e., acetate and ethanol) production is reliable and applicable at technology readiness level (TRL) 7, as in the case of bioethanol commercialization by LanzaTech (Pacheco et al., 2023). However, the economic feasibility decreases when the

Table 1
Properties of syngas fermentation process.

Process	Description
Upstream	Isolation and characterization of biocatalysts (microbial strains) Strain development for improved product valorization and productivity
Midstream	Optimization of substrate supply efficiency Medium optimization for improved process economics Development of operational strategies for enhanced productivity and product stability
Downstream	Product purification and commercialization

product with an increased degree of reduction is changed to the target product, due to the lack of development of the upstream process. As will be described later, acetogens, which are key biocatalysts for syngas fermentation, mainly produce C2 chemicals and usually lack ability to produce chemicals with high degree of reduction. Therefore, it is necessary to develop upstream phase beyond the current level. Key factors will be investigated and strain development strategies for product valorization will be summarized herein.

2. Acetogenesis

2.1. Acetogens

Drake (1995) defined acetogens as obligately anaerobic bacteria capable of synthesizing acetyl-CoA, cell carbon and energy from CO_2 by the Wood-Ljungdahl pathway (WLP). Most acetogens can utilize a wide range of substrates from heterotrophic substrates (e.g., glucose and fructose) to other C1 compounds (e.g., CO and methanol), enabling their growth on various industrial waste gases, including those produced from steel manufacturing, oil refining, natural gas and coal (Drake, 1995; Müller, 2003; Ragsdale and Pierce, 2008). They are commonly referred to as "homoacetogens," since the majority of acetogens produce only acetate as a result of C1 gas utilization. The first isolated strain, *Clostridium aceticum,* was identified by Wieringa (1936) but was not studied until 1981 due to culture loss (Braun et al., 1981). *Moorella thermoacetica* (f. *C. thermoaceticum*), the second isolated strain by Fontaine et al. (1942), was used as a model strain in the study of acetogenic metabolism. Over 100 acetogenic species (in 29 genera), including *C. aceticum* and *M. thermoacetica,* are currently known (Table 2) (Abubackar et al., 2011; Drake et al., 2008; Lee et al., 2022; Takors et al., 2018). They have been found in various habitats, such as the gastrointestinal tract, soils, oil fields, sewage sludge and freshwater sediments. Acetogens exhibit a broad range of pH and temperature tolerance, making them adaptable to diverse environmental conditions. These anaerobes use H_2:CO_2 as a primary substrate, although some strains can also utilize other C1 chemicals such as CO and methanol. For instance, various strains including *Acetobacterium bakii*, *A. tundrae*, *Alkalibaculum bacchi*, *Blautia producta*, *Butyribacterium methylotrophicum*, *C. autoethanogenum*, *C. carboxidivorans*, *C. ljungdahlii*, *C. ragsdalei*, *E. limosum* and *M. thermoacetica* have been shown to utilize CO (Abrini et al., 1994; Allen et al., 2010; Chang et al., 1997; Geerligs et al., 1987; Jiang et al., 2009; Kang et al., 2019; Kotsyurbenko et al., 1995; Kundiyana et al., 2010; Liou et al., 2005; Lynd et al., 1982; Simankova et al., 2000; Tanner et al., 1993). Methanol utilization has been observed in *A. bakii*, *A. dehalogenans*, *A. paludosum*, *A. tundrae*, *A. woodii*, *A. bacchi*, *B. methylotrophicum*, *C. formicaceticum*, *E. aggregans*, *E. limosum*, *M. mulderi*, *M. thermoacetica*, *Sporomusa acidovorans*, *S. aerivorans*, *S. intestinalis*, *S. ovata*, *S. paucivorans* and *S. sphaeroides* (Allen et al., 2010; Balk et al., 2003; Boga et al., 2003; Gottschalk and Braun, 1981; Hattori et al., 2013; Hermann et al., 1987; Kim et al., 2021; Kotsyurbenko et al., 1995; Mechichi et al., 1998; Möller et al., 1984; Ollivier et al., 1985; Seifritz et al., 1993; Simankova et al., 2000; Traunecker et al., 1991; Zeikus et al., 1980). To better understand the metabolism of acetogens and to develop strains with specific properties, researchers have completed whole-genome sequencing for over half of all known acetogens. Furthermore, genetic engineering tools have been developed for a limited number of strains, such as *A. woodii*, *C. autoethanogenum*, *C. ljungdahlii*, *E. limosum*, *M. thermoacetica* and *Thermoanaerobacter kivui* (Basen et al., 2018; Bengelsdorf et al., 2016; Cheng et al., 2019; Huang et al., 2016; Jeong et al., 2020; Kita et al., 2013; Liew et al., 2017; Mock et al., 2015; Nagaraju et al., 2016; Philipps et al., 2019; Rahayu et al., 2017; Schoelmerich et al., 2018; Shin et al., 2019; Strätz et al., 1990; Tremblay et al., 2012; Wang et al., 2021).

2.2. Wood-Ljungdahl pathway (WLP)

As indicated by the name "acetogen", these microorganisms are known for their acetate-forming abilities via acetogenesis, which is distinct from acid-forming microbial reactions (acidogenesis). The Wood-Ljungdahl Pathway (WLP) is the key metabolic pathway for acetogenesis, and while some acetogens can use various C1 chemicals such as CO or methanol via the WLP, the following pathway description focuses on CO_2 as a carbon source coupled with H_2 as an electron source.

As shown in Fig. 1, CO_2 is incorporated into the WLP through two branches: the methyl and carbonyl branches (Drake et al., 2008; Schuchmann and Müller, 2014; Shively and Barton, 1991; Wood, 1991). The methyl branch begins with the reduction of CO_2 to formate by formate dehydrogenase (Fdh). Formate is then activated to formyl-tetrahydrofolate (THF) by formyl-THF synthetase (Fhs), which requires one ATP. Formyl-THF is further metabolized to methenyl-THF, methylene-THF and finally methyl-THF through a series of reactions catalyzed by formyl-THF cyclohydrolase (Fch), methylene-THF dehydrogenase (Mthfd) and methylene-THF reductase (Mthfr). In the final step of the methyl branch, the methyl group is transferred to a corrinoid iron-sulfur-containing protein (CoFeSP). This series of enzymatic reactions requires six electrons (Diekert, 1990; Drake and Daniel, 2004). The carbonyl branch begins with the reduction of CO_2 to CO by CO dehydrogenase/acetyl-CoA synthase (CODH/ACS), using two electrons (Diekert, 1990; Diekert and Thauer, 1978; Drake et al., 1980). The bifunctional CODH/ACS also catalyzes the condensation of CO with methyl-CoFeSP to form a C2 chemical (acetyl-CoA) from a C1 chemical.

During the reduction of CO_2, a total of eight electrons and one ATP are required. Since CO_2 is a fully oxidized molecule, a source of reducing equivalents must be provided. One common strategy for acquiring these reducing equivalents is the electron bifurcation of H_2. The electron-bifurcating hydrogenase complex can oxidize H_2 to 2 H^+, which in turn reduces ferredoxin (Fd) and NAD^+. Acetate production is a means of generating ATP, requiring only two steps (Andreesen et al., 1973). Firstly, phosphate acetyltransferase (Pta) catalyzes the conversion of acetyl-CoA to acetyl-phosphate (acetyl-P) without the need for reducing equivalents or ATP hydrolysis. Secondly, acetate kinase (Ack) converts acetyl-P to acetate, thereby producing one ATP. As a result, the net ATP yield from the pathway is zero (Poehlein et al., 2012; Trischler et al., 2022). In summary, the WLP-mediated acetogenesis reaction can be represented by the following equation: $4\ H_2 + 2\ CO_2 \rightarrow CH_3COOH + 2\ H_2O$ ($\Delta G^{0'} = -95$ kJ/mol).

2.3. Energy conservation

Acetogens possess a chemiosmotic energy conservation (CEC) system, which comprises an Fd-driven ion pump and ATP synthase for ATP synthesis (Schoelmerich et al., 2019). The ion pump can be classified into two types based on their final electron acceptor (Fig. 1 and Table 2). The Ech (energy-converting hydrogenase) complex, first discovered by Ljungdahl (1995), catalyzes the transfer of electrons from Fd^{2-} produced by the hydrogenase (Section 2.2) to H^+ to establish a transmembrane H^+ gradient. On the other hand, the Rnf (*Rhodobacter* nitrogen fixation) complex, an ion-translocating Fd:NAD^+ oxidoreductase, is another type of redox-driven ion pump for chemiosmotic gradient. The complex serves as an energy-coupled transhydrogenase, balancing Fd^{2-} requirements with NAD^+ and generating a transmembrane H^+ or Na^+ gradient (Hess et al., 2016, 2013; Köpke et al., 2010; Tremblay et al., 2012). The ion gradient established by either the Ech or Rnf complex is subsequently utilized for ATP synthesis (Heise et al., 1993, 1991; Mock et al., 2015). Since net ATP generated via the WLP is zero (Section 2.2), the CEC system is responsible for the majority of ATP production during C1 gas fermentation (the term "fermentation" is used because the final electron acceptor is endogenous NAD^+).

Table 2
List of acetogens discovered to date and their characteristics.

Strain	Temp (℃)	pH	Source of isolate	Genome sequencing	Type of CEC	Product
Homoacetogenic strain						
Acetoanaerobium noterae	37	7.6–7.8	Sediment	GCA_900168025	Rnf	Acetate
Acetobacterium bakii	20	6.5	Sediment	GCA_900562175	Rnf	Acetate
A. dehalogenans	25	7.3–7.7	Sludge	GCA_000472665	Rnf	Acetate
A. fimetarium	30	7.5	Sediment	GCA_014284475	Rnf	Acetate
A. paludosum	20	7.0	Sediment	GCA_008086595	Rnf	Acetate
A. tundrae	20	7.0	Tundra	GCA_008086615	Rnf	Acetate
A. wieringae	30	7.6	Sludge	CP087994	Rnf	Acetate
A. woodii	30	7.0–7.4	Mud	CP002987	Rnf	Acetate
Acetohalobium arabaticum	37	7.6–8.0	Lake	CP002105	Rnf	Acetate
Blautia hydrogenotrophica	35–37	6.6	Feces	GCA_025289255	Rnf	Acetate
B. schinkii	39	6.5–7.0	Rumen	GCA_000702025	Rnf	Acetate
B. producta	37	6.5–6.8	Sludge	CP039126	Rnf	Acetate
Caloramator fervidus	68	7.0–7.5	Thermal spring	GCA_900108045		Acetate
Clostridium bovifaecis	30	7.0	Feces	CP046522	Rnf	Acetate
C. magnum	30–32	7.0	Freshwater	GCA_900129955	Rnf	Acetate
C. scatologenes	25–30	5.8–6.9	Sediment	CP009933	Rnf	Acetate
Desulfosporomusa polytropa	28	6.1–8.2	Sediment	–	–	Acetate
Fuchsiella alkaliacetigena	40	8.8–9.3	Lake	GCA_023227765	Rnf	Acetate
F. ferrireducens	30–37	9.8	Lake	–	–	Acetate
Holophaga foetida	28–32	6.8–7.5	Mud	GCA_000242615		Acetate
Marvinbryantia formatexigens	37	7.0	Feces	CP102268	Rnf	Acetate
Moorella glycerini	58	6.3–6.5	Mixed sediment–water	CP046244	Ech	Acetate
M. mulderi	65	7.0	Sludge	GCA_001594015	Ech	Acetate
M. thermoacetica	55–60	5.7–6.8	Feces	CP012370	Ech	Acetate
Natroniella acetigena	37	9.7–10.0	Mud	GCA_023227745	Rnf	Acetate
N. histidinovorans	37–40	9.4	Sediment	–	–	Acetate
N. sulfidogena	35	9.8–10.0	Lake	GCA_023223645	Rnf	Acetate
Schnuerera ultunensis	37	7.0	Feces	LT669839	Rnf	Acetate
Sporanaerobacter acetigenes	40	7.4	Sludge	GCA_900130025	Rnf	Acetate
Sporomusa acidovorans	35	6.5	Wastewater	GCA_002257695	Rnf	Acetate
S. aerivorans	30	7.0	Gut	–	–	Acetate
S. intestinalis	35–37	7.0	Gut	–	–	Acetate
S. malonica	28–32	7.3	Sediment	GCA_900176355	Rnf	Acetate
S. ovata	34–39	5.3–7.2	Animal feed	GCA_000423685	Rnf	Acetate
S. paucivorans	35	7.0	Sediment	–	–	Acetate
S. silvacetica	30	6.8	Soil	GCA_002257705	Rnf	Acetate
S. termitida	30	7.2	Gut	CP036259	Rnf	Acetate
Terrisporobacter mayombei	33	7.3	Gut	GCA_020748465	Rnf	Acetate
Thermacetogenium phaeum	58	6.8	Wastewater	CP003732		Acetate
Thermoanaerobacter kivui	66	6.4	Lake	CP009170	Ech	Acetate
Tindallia californiensis	37	9.5	Lake	GCA_900107405	Rnf	Acetate
Treponema primitia	30	7.2	Gut	CP001843	Rnf	Acetate
Non-homoacetogenic strain						
Acetitomaculum ruminis	38	6.8	Rumen	GCA_900112085	Rnf	Acetate, propionate, isobutyrate, butyrate, isovalerate, valerate
Acetonema longum	30–33	7.8	Gut	GCA_000219125		Acetate, butyrate
Alkalibaculum bacchi	37	8.0–8.5	Soil	GCA_003317055	Rnf	Acetate, ethanol
Butyribacterium methylotrophicum[a,b]	37–40	7.5	Sludge	–	–	Acetate, butyrate
Clostridioides difficile	37	7.0	Feces	GCA_000438845	Rnf	Acetate, ethanol
Clostridium aceticum	30	8.3	Soil	CP009687	Rnf	Acetate, ethanol
C. autoethanogenum[a,c]	37	5.8–6.0	Feces	CP012395	Rnf	Acetate, ethanol, butanediol
C. carboxidivorans	38	6.2	Lake	CP011803	Rnf	Acetate, ethanol, butyrate, butanol
C. drakei	25–30	3.6–6.8	Peat	CP020953	Rnf	Acetate, butyrate
C. formicaceticum	37	7.2	Sludge	CP020559	Rnf	Acetate, formate
C. ljungdahlii	37	6.0	Animal waste	CP001666	Rnf	Acetate, ethanol, butanediol
C. ragsdalei[a]	35	6.0	Sediment	GCA_001675165	Rnf	Acetate, ethanol,butanediol
Eubacterium aggregans	35	7.2	Wastewater	GCA_900107815	Rnf	Acetate, formate
E. limosum	37	7.0	Anaerobic digester	CP002273	Rnf	Acetate, butyrate
E. maltosivorans	35–37	7.0–7.5	Feces	CP029487	Rnf	Acetate, butyrate
Natranaerofaba carboxydoora	48–50	9.5	Sediment	GCA_022539405	Rnf	Acetate, formate
Oxobacter pfennigii	36–38	7.3	Rumen fluid	GCA_001317355	Rnf	Acetate, butyrate
Proteocatella sphenisci	29	8.3	Guano	GCA_000423525	Rnf	Acetate, ethanol, butyrate
S. sphaeroides	35–39	6.4–7.6	Mud	GCA_023660125	Rnf	Acetate, ethanol
Terrisporobacter glycolicus	22–37	7.4–7.6	Mud	GCA_000439105	Rnf	Acetate, ethanol
Treponema azotonutricium	30		Gut	CP001841	Rnf	Acetate, ethanol

[a] Not validly published name. The strain needs for taxonomic reclassification; [b]taxonomically very close to *E. callanderi*; [c]taxonomically very to *C. ljungdahlii*. -: No genome information.

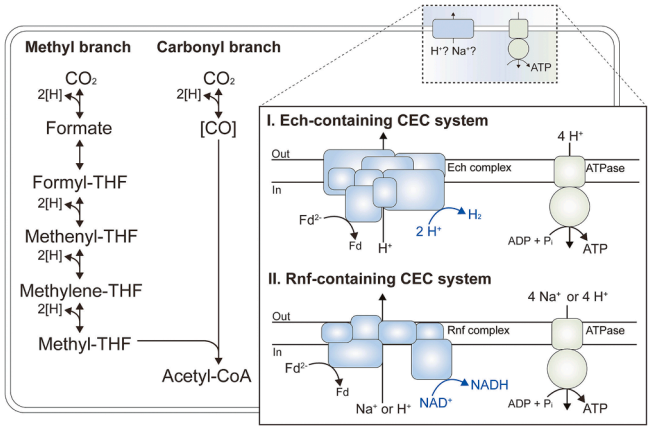

Fig. 1. The Wood-Ljungdahl pathway (WLP) and chemiosmotic energy conservation (CEC) system in acetogens. Ech and Rnf complexes were drawn with reference to Hess et al. (2013) and Hess et al. (2014).

3. Biochemicals from syngas components

3.1. Chain elongation

Although acetate is the primary product of acetogens, some acetogens can also produce another C2 chemical, such as ethanol or chain-elongated chemicals (Arslan et al., 2021; Mutyala and Kim, 2022). Chain-elongated chemicals that can be natively synthesized are basically limited to butyrate, butanol and butanediol (Table 2). Since the primary metabolites of acetogens result from the condensation of acetyl-CoA, odd-numbered carbon compounds (e.g., C3) are rarely produced. The production of chemicals higher than C4 is also difficult due to the required amounts of reducing equivalents. Therefore, the focus is on C2 and C4 production in this section. Product formation can be divided into two types based on the building blocks for assimilation. This division is important as energy and product yield may vary depending on the pathway preferred by the acetogens. Fig. 2 illustrates the C2 and C4 product formation pathways that have been reported in acetogens.

Ethanol can be synthesized from acetyl-CoA through two reduction steps involving acetaldehyde. The reduction of acetyl-CoA to ethanol using NAD(P)H as an electron donor is catalyzed by aldehyde dehydrogenase (Ald) and mono-/bi-functional alcohol dehydrogenase (Adh) (Extance et al., 2013; Goodlove et al., 1989; Peng et al., 2008). Conversely, C4 production requires a more complex process and higher

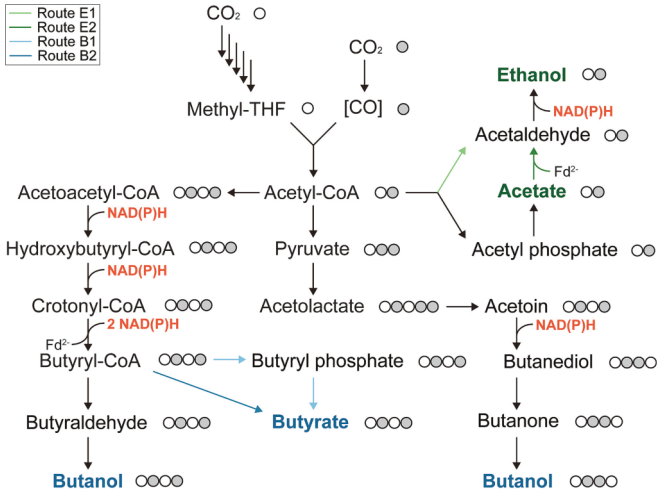

Fig. 2. Acid and alcohol production from CO_2. Open and closed circles represent carbon from methyl and carbonyl branch, respectively.

NAD(P)H production than those of aceto/ethanologenesis. Butyrate/butanol formation begins with two moles of acetyl-CoA, which are condensed to acetoacetyl-CoA by thiolase (Thl). Acetoacetyl-CoA is subsequently reduced to hydroxybutyryl-CoA by 3-hydroxybutyryl-CoA dehydrogenase (Hbd) and then dehydrated to crotonyl-CoA by crotonase (Crt). These reduction and dehydration steps require one NAD(P)H, respectively. Crotonyl-CoA and 2 NAD(P)H are reduced to butyryl-CoA and one Fd^{2-}, respectively, by an electron-bifurcating butyryl-CoA dehydrogenase (Bcd/EtfAB complex) (Li et al., 2008). Butyryl-CoA is a key intermediate in the production of butyrate and butanol. Phosphotransbutyrylase (Ptb) converts butyryl-CoA to butyryl phosphate, which is used as a precursor for butyrate formation by butyrate kinases (Ack or Buk). Alternatively, two dehydrogenases (Ald and Adh) catalyze the reaction for butanol production. In the metabolic pathway from pyruvate to butanediol, the initial conversion is catalyzed by acetolactate synthase, which links two pyruvates to acetolactate. Then, acetolactate is split into acetoin and CO_2 by an acetolactate decarboxylase. Finally, acetoin is reduced to butanediol by the action of butanediol dehydrogenase using one NAD(P)H (Köpke et al., 2011). Acetate can also serve as a building block for the production of ethanol and butyrate. Aldehyde:ferredoxin oxidoreductase (AOR) catalyzes the reversible reduction of an acetate to acetaldehyde, consuming an Fd^{2-} as an electron donor (Joshi and Mishra, 2022). Adh can then further reduce acetaldehyde to ethanol. Acetate-assimilatory butyrate production follows the same pathway, with the formation of butyryl-CoA by acetyl-CoA condensation, but CoA transferase is used instead of Ptb in the later step (Bortolucci et al., 2023; Gabris et al., 2015; Park et al., 2017).

3.2. Reducing equivalents and redox cycle

The reducing equivalents involved in acetogenesis are Fd^{2-} and NAD(P)H. Fd^{2-} is primarily consumed for electron transfer and CO_2 reduction in both branches of the WLP. On the other hand, NAD(P)H is responsible for most intracellular redox reactions, including reduced-end product (i.e., butanol, butyrate and butanediol) formation, except acetate. Therefore, NAD(P)H is pertinent for chemical production (Fig. 2), and its yield may vary depending on the strains and the chemicals produced. Previously, Schuchmann and Müller (2014) classified acetogens into three groups according to their CEC type: the Rnf-containing, Na^+-dependent (Group 1), the Rnf-containing, H^+-dependent (Group 2) and the Ech-containing, H^+-dependent (Group 3). The NAD(P)H generation using this classification is explained herein.

All acetogens possess a hydrogenase complex, which is involved in electron bifurcation as an initial step for NADH generation. In this hydrogenase complex, two H_2 molecules are oxidized to four H^+ ions, and the four electrons from H_2 are split and transferred to Fd and NAD^+ (Katsyv et al., 2023). This process leads to the formation of two Fd^{2-} and two NADH molecules through electron bifurcation. The WLP is also present in all strains. Since the enzymes involved in this pathway are reversible, NAD(P)H can be obtained through the reverse reaction of the WLP. However, the yield of NAD(P)H varies depending on the Fdh type. Strains containing an electron-bifurcating *fdh-hyd* complex, such as *E. limosum* KIST612 (reclassified as *E. callanderi* KIST612), *C. autoethanogenum* DSM 10061 and *C. ljungdahlii* DSM 13528, obtain 3.5 NAD(P)H (Wang et al., 2013). This yield is half that which could be obtained by strains with a general type of Fdh for NAD(P)H production (e.g., *M. thermoacetica* ATCC 39073). In the case of *A. woodii* DSM 1030, only two NADH molecules are generated due to its H_2-dependent CO_2 reductase (Schuchmann and Müller, 2013). The final difference is caused by the presence or absence of Fd:$NAD(P)^+$ oxidoreductase. Group 1, which has the Rnf complex, transfers electrons from Fd^{2-} to NAD^+, generating NADH (Section 2.3). Group 2 has both the Rnf and the Nfn complexes (Fd:$NADP^+$ oxidoreductase). Therefore, NADH and NADPH can be obtained by the Rnf and the Nfn, respectively. Group 3 only obtains NADPH through the oxidation of Fd^{2-} and NADH because there is only Nfn and no Rnf (Wang et al., 2010). To aid in understanding

what has been explained so far, Fig. 3 and Table 3 shows the reducing equivalents balance and consequent ATP depending on the acetogen group under the H_2:CO_2 condition.

3.3. Biochemical producibility

Based on the information in Sections 3.1 and 3.2, the maximal ATP yield per substrate carbon used for each product was calculated theoretically (Table 4). The producibility of each chemical varies depending on the substrate used. Under the H_2:CO_2 condition, it is predicted that all groups can produce not only acetate but also butyrate. Additionally, acetogens can indirectly produce ethanol through acetate assimilation (Route E2 in Fig. 2), except for Group 2. Butanol production is only predicted in Groups 1[b] and 3. Although it is possible that all products can be produced under the CO condition, strains belonging to Group 1[a] are deemed inappropriate for biochemical production due to the inhibition of H_2-dependent CO_2 reductase by CO (Bertsch and Müller, 2015; Ceccaldi et al., 2017).

Butyrate is predicted to be the most energetically favorable product except acetate. Specifically, acetate-assimilatory butyrate production may be a key route to consider when assessing ATP yield. However, it has been reported that butyrate is not only a minor product of CO fermentation but also cannot be produced by H_2:CO_2 (Kim et al., 2021; Litty and Müller, 2021; Park et al., 2017). This suggests that the product spectrum may be determined by metabolic flux and the NADH/NAD^+ ratio rather than being solely an issue of energetics. Although limited research has been conducted in this regard, Shen et al. (1996) reported that butyrate production started when the NADH/NAD^+ ratio was 0.5 or higher in *B. methylotrophicum*. As the solubility of H_2 is much lower than that of CO, the H_2:CO_2 condition may pose challenges for NADH accumulation. Therefore, almost all substrates could be directly converted to acetate under the H_2:CO_2 condition. Unlike H_2 and CO_2, the CO condition can be relatively advantageous for NADH accumulation, resulting in minor butyrate production. Indirect ethanol production is also energetically preferable, as the ATP yield from ethanol production is the same or almost equal to the yield from acetate production. Unlike butyrate production, ethanol production requires only 1–2 reducing equivalents, making it a potential major product along with acetate.

Butyrate formation has been identified in specific bacterial strains, including *Acetonema longum*, *B. methylotrophicum*, *C. carboxidivorans*, *C. drakei*, *C. scatologenes* and *E. limosum* (Chang et al., 1997; Grethlein et al., 1991; Kane and Breznak, 1991; Liou et al., 2005; Maddipati et al., 2011). Butanol and butanediol are produced by *Clostridium* spp.

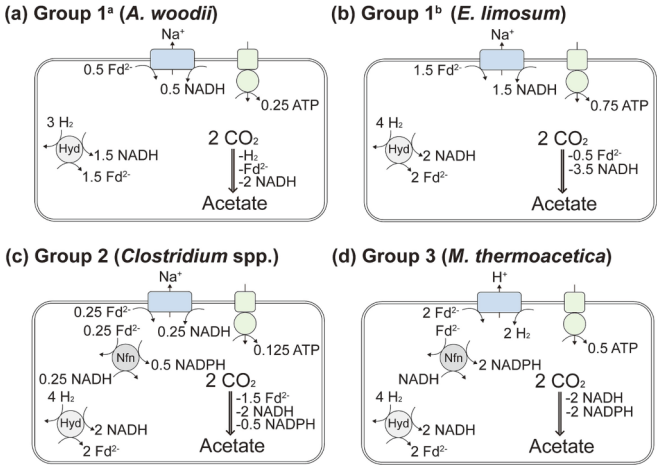

Fig. 3. Group-dependent acetogenesis under the H_2/CO_2 condition. A model strain of each group is selected as (a) *A. woodii* DSM 1030, (b) *E. limosum* KIST612, (c) *C. autoethanogenum* DSM 10061, and (d) *M. thermoacetica* ATCC 39073.

Table 3
Reducing equivalents balance and consequent ATP production based on the classification of acetogens under the H_2/CO_2 condition.

	Group 1[a]	Group 1[b]	Group 2	Group 3
Electron-bifurcating hydrogenase complex				
Fd^{2-}	+1.50	+2.00	+2.00	+2.00
NADH	+1.50	+2.00	+2.00	+2.00
NADPH	0.00	0.00	0.00	0.00
ATP	0.00	0.00	0.00	0.00
The Wood-Ljungdahl pathway				
Fd^{2-}	−1.00	−0.50	−1.50	0.00
NADH	−2.00	−3.50	−2.00	−2.00
NADPH	0.00	0.00	−0.50	−2.00
ATP	0.00	0.00	0.00	0.00
Transhydrogenase complex				
Fd^{2-}	0.00	0.00	−0.25	−1.00
NADH	0.00	0.00	−0.25	−1.00
NADPH	0.00	0.00	+0.50	+2.00
ATP	0.00	0.00	0.00	0.00
Chemiosmotic energy conservation				
Fd^{2-}	−0.50	−1.50	−0.25	−1.00
NADH	+0.50	+1.50	+0.25	0.00
NADPH	0.00	0.00	0.00	0.00
ATP	+0.25	+0.75	+0.13	+0.50

[a]Acetogens containing hydrogen-dependent CO_2 reductase; [b]acetogens containing electron-bifurcating *fdh-hyd* complex.

However, only a few strains among the C4-producing acetogens have been tested for industrialization (Table 5). *B. methylotrophicum* ATCC 33266, *C. carboxidivorans* P7 and *E. limosum* KIST612 were used for butyrate production. It should be noted that butyrate was not produced under the H_2:CO_2 condition. Instead, CO or H_2:CO:CO_2 was added as the carbon and energy source. Interestingly, H_2:CO:CO_2 has been found to be more favorable for butyrate production than CO (Kim et al., 2021; Vees et al., 2022). For instance, *E. limosum* KIST612 produced butyrate at 0.1 g/L under the CO condition, whereas the production was 0.2 g/L under the H_2:CO:CO_2 condition (Kim et al., 2021; Park et al., 2017). This could be due to the oxidation of H_2 by the hydrogenase complex, which generates additional NADH for butyrate production. It is noteworthy that the carbon flow of C4-producing acetogens in Group 2 diverges in multiple directions compared to Group 1. Therefore, Group 1 appears to be more advantageous for butyrate production than Group 2. *C. carboxidivorans* P7, which belongs to Group 2, produced butyrate at a lower rate (0.1 g/L) than that of Group 1 (*B. methylotrophicum* ATCC 33266: 6.0 g/L; *E. limosum* KIST612: 0.2 g/L) (Kim et al., 2021; Shen et al., 2017; Worden et al., 1989). Additionally, *C. carboxidivorans* P7 has been used for both butanol and butyrate production. The strain produced butanol at 2.3 g/L from CO (Fernández-Naveira et al., 2016). Butanediol production has also been attempted using *C. ljungdahlii* DSM 13528 and *C. autoethanogenum* DSM 23693. The strains produced butanediol at 0.5 and 0.4 g/L under H_2:CO and H_2:CO:CO_2 conditions, respectively (Jack et al., 2019).

Some strains have been genetically engineered to enable the hyperproduction of native chemicals, such as acetate, as well as the production of non-native chemicals with a high degree of reduction. For instance, the butyrate production pathway has been introduced into the non-butyrate-producing strains *A. woodii* DSM 1030 and *C. ljungdahlii* DSM 13528. Although *A. woodii* DSM 1030 could not produce as much butyrate as the native butyrate-producing strain, butyrate production was confirmed in the genetically engineered strain (Chowdhury et al., 2022). Engineered DSM 13528 strains hold promise as a "good" butyrate producer, yielding 0.8 g/L of butyrate, which exceeds the butyrate titers of *C. carboxidivorans* P7 and *E. limosum* KIST612 (Ueki et al., 2014). Non-native butanol production has been attempted in *C. ljungdahlii* and *C. autoethanogenum*. In the first trial, *thlA*, *hbd*, *crt*, *bcd*, *adhE* and *bdhA* were introduced into *C. ljungdahlii*. Although the developed strain could

Table 4
Theoretical maximal ATP yield depending on the substrate and the target product.

Substrate	Product	Stoichiometry	ATP/carbon
H_2/CO_2	Acetate	$2\ CO_2 + 4\ H_2 \rightarrow C_2H_4O_2$	Group 1[a]: 0.13 Group 1[b]: 0.38 Group 2: 0.06 Group 3: 0.25
	Ethanol[d]	$2\ CO_2 + 6\ H_2 \rightarrow C_2H_6O$	Group 1[a]: 0.13 Group 1[b]: 0.38 Group 3: 0.25
	Butyrate[e]	$4\ CO_2 + 11\ H_2 \rightarrow C_4H_8O_2$	Group 1[a]: 0.19 Group 1[b]: 0.44 Group 2: 0.13 Group 3: 0.31
	Butyrate[f]	$4\ CO_2 + 11\ H_2 \rightarrow C_4H_8O_2$	Group 1[a]: 0.27 Group 1[b]: 0.35 Group 2: 0.23 Group 3: 0.40
	Butanol	$4\ CO_2 + 11\ H_2 \rightarrow C_4H_{10}O$	Group 1[b]: 0.19 Group 3: 0.06
CO	Acetate	$4\ CO \rightarrow C_2H_4O_2 + 2\ CO_2$	Group 1[b]: 0.44 Group 2: 0.28 Group 3: 0.38
	Ethanol[c]	$5\ CO \rightarrow C_2H_6O + 3\ CO_2$	Group 1[b]: 0.25 Group 2: 0.13 Group 3: 0.20
	Ethanol[d]	$6\ CO \rightarrow C_2H_6O + 4\ CO_2$	Group 1[b]: 0.38 Group 2: 0.27 Group 3: 0.33
	Butyrate[e]	$11\ CO \rightarrow C_4H_8O_2 + 7\ CO_2$	Group 1[b]: 0.41 Group 2: 0.30 Group 3: 0.36
	Butyrate[f]	$11\ CO \rightarrow C_4H_8O_2 + 7\ CO_2$	Group 1[b]: 0.45 Group 2: 0.32 Group 3: 0.40
	Butanol	$11\ CO \rightarrow C_4H_8O_2 + 7\ CO_2$	Group 1[b]: 0.32 Group 2: 0.20 Group 3: 0.27
	Butanediol	$13\ CO \rightarrow C_4H_{10}O + 9\ CO_2$	Group 1[b]: 0.21 Group 2: 0.07 Group 3: 0.15

[a]acetogens containing hydrogen-dependent CO_2 reductase; [b] acetogens containing electron-bifurcating *fdh-hyd* complex; [c] ethanol production through Route E1 as shown in Fig. 2; [d] ethanol production through Route E2 as shown in Fig. 2; [e] butyrate production through Route B1 as shown in Fig. 2; [f] preferential butyrate production through Route B2 as shown in Fig. 2.

produce butanol, it did not match the level of production achieved by native butanol producers (Dürre, 2016). Subsequent modifications involving the introduction of *etfAB*, *ptb* and *buk*, which are involved in the butyrate production pathway, as well as *aor* in *C. autoethanogenum,* induced butyrate production (0.3 g/L) but greatly improved butanol production to 1.9 g/L (Dürre, 2016). The additional ATP obtained through butyrate production may have contributed to the improved butanol production.

4. Strategies for product valorization

4.1. Carbon flux control in strain metabolism

Genetic or metabolic engineering for carbon redirection toward C4 chemicals has not been extensively investigated in acetogens. Generally, metabolic engineering studies employing strains such as *Escherichia coli* and *Corynebacterium* spp. (those are common host microbes for biochemical production) have tended to focus on suppressing competing pathways for biochemical production. However, Ueki et al. (2014) not only enabled non-native butyrate production in *C. ljungdahlii* but also improved the ability of the strain to direct the carbon flow toward butyrate formation by knocking out the native alcohol and acetate production pathways. In a previous study, a butyrate titer of 1.5 g/L (equivalent to 70% of substrate carbon) was confirmed under the CO: CO_2 condition, indicating that a significant amount of carbon was transferred to butyrate. Although under heterotrophic conditions, Baur et al. (2022) also showed the feasibility of the approach through hyper butyrate production improved from about 3.3 to up to 5.9 times in genetically modified strains. Alternatively, enhancing the native C4-producing pathway may also be a viable approach. Wang et al. increased butyrate production by 1.3-fold through overexpression of the butyrate pathway in *B. methylotrophicum* ATCC 33266 (Wang et al., 2021).

4.2. Way of cultivation and mixotrophy

Syngas fermentation is generally conducted using pure culture cultivation. However, (homo)acetogens which are produce C2 as (only or) major products, have a fundamental metabolic limitation (lack of required NADH) in valorized chemical production. There are some approaches of “mixed cultivation” using acetogens for C1 gas conversion and non-acetogens for product valorization in order to have symbiotic

Table 5
Biochemical production from C1 gas in wild-type and genetically engineered acetogens.

Product	Strain	Genotype	Substrate	Titer (g/L)
Butyrate	*A. woodii* DSM 1030	P_{pta}-*ack-thl-hbd-crt-bcd-etfB-etfA-ptb*	CO_2/methanol	0.0
	B. methylotrophicum ATCC 33266	Wild-type	CO	6.0
		Wild-type	CO_2/methanol	2.0
		P_{thl}-*atoB-paaH-crt-bcd*	CO_2/methanol	2.6
	C. autoethanogenum	P_{pta}-*thlA-crt-bcd-etfB-etfA-hbd ptb buk aor bdh*	CO	0.3
	C. carboxidivorans P7	Wild-type	$H_2/CO/CO_2$	0.1
		Wild-type	$H_2/CO/CO_2$/glucose	0.6
	C. ljungdahlii DSM 13528	P_{pta}-*thl-crt-bcd-etfA-etfB-hbd* P_{pta}-*ptb-buk*	CO/CO_2	0.8
		P_{pta}-*thl-crt-bcd-etfA-etfB-hbd* P_{pta}-*ptb-buk Δpta ΔadhE1 Δctf*	CO/CO_2	1.5
	E. limosum KIST612	Wild-type	CO	0.1
		Wild-type	$H_2/CO/CO_2$	0.2
		Wild-type	CO/acetate	0.5
		Wild-type	$H_2/CO/CO_2$/methanol	0.6
Butanol	*C. autoethanogenum*	P_{pta}-*thlA-crt-bcd-etfB-etfA-hbd ptb buk aor bdh*	CO	1.9
	C. carboxidivorans P7	Wild-type	CO	2.3
		Wild-type	CO/glucose	2.6
	C. ljungdahlii DSM 13528	*thlA hbd crt bcd adhE bdhA*	CO/CO_2	0.2
Butanediol	*C. autoethanogenum* DSM 23693	Wild-type	$H_2/CO/CO_2$	0.4
	C. autoethanogenum LZ1561	Wild-type	$H_2/CO/CO_2$	1.1
	C. ljungdahlii DSM 13528	Wild-type	H_2/CO	0.5
	Clostridium sp. MBD136	*pta*$^-$ *ack*$^-$ *spo0A*$^-$ *spo0J*$^-$ *fdh*$^+$ *pfl*$^+$ *als*$^+$ *ald*$^+$ *adh*$^+$	$H_2/CO/CO_2$	9.2

Table 6
Variation of chemical production by changing operating parameters in the same system.

Strain	Culture design	Substrate	Titer (g/L)	Temp (℃)	pH
E.limosum KIST612	BCR	CO/CO_2	Acetate: 4.9 Butyrate: 1.3	37	7
	BCR	CO/CO_2	Acetate: 7.0 Butyrate: 1.7	37	7
	BCR	CO/CO_2	Acetate: 8.1 Butyrate: 1.3	37	7
	BCR	CO/CO_2	Acetate: 9.8 Butyrate: 6.7	37	7
C. carboxidivorans P7	GLR	$CO/CO_2/H_2/N_2$	Ethanol: 1.5 Butanol: 0.4	37	6
	STR	$CO/CO_2/H_2/N_2$	Ethanol: 1.2 Butanol: 0.8	37	6
	STR	CO/CO_2	Acetate: 1.8 Ethanol: 6.3 Butanol: 0.8 Butyrate: 0.2	33	5–6

BCR: bubble column reactor; GLR: gas-lift loop reactor; STR: stirred tank reactor.

effect of both. Cha et al. (2021) reported CO conversion to 3-hydroxypropionate and itaconate using *E. limosum* and engineered *E. coli*.

In addition, "mixotrophy" referred to at least two types of major carbon and energy sources used in single culture, may be a way to enhance C4 production. Energy-rich compounds, such as glucose or fructose, are typically selected as the substrate for mixotrophy. *C. carboxidivorans* P7 exhibited a 6.2- and 1.1-fold increase in butyrate and butanol production, respectively, compared to C1 gas conditions when glucose was used as a substrate (Vees et al., 2022). Acetate assimilation via CoA transferase suggests that acetate has the potential for mixotrophy. Park et al. (2017) confirmed that *E. limosum* KIST612 produced 0.5 g/L of butyrate, which was a five-fold improvement over production with only CO through mixotrophy with CO and acetate. Recently, mixotrophy using methanol has been attempted. Methanol is an attractive substrate for C4 chemical production, as it rapidly generates three NADH through the oxidation of one methanol molecule (Kremp et al., 2018). Increased butyrate production using methanol has been reported in engineered *A. woodii*, *B. methylotrophicum* ATCC 33266 and *E. limosum* KIST612 (Chowdhury et al., 2022; Kim et al., 2021; Wang et al., 2021).

4.3. Process control

In gas fermentation utilizing synthetic gas as the substrate, several critical parameters affect the product profile (He et al., 2022; Sun et al., 2019). Common parameters related to the culture environment in general fermentation (carbohydrate or gas fermentation) processes are pH, temperature and medium composition. These parameters affect the type of the end product and productivity. Moreover, gas fermentation employs gases as the substrate, which heightens the importance of factors such as mass transfer rate and dissolved gas concentration that affect how well the gas substrate can be delivered to the microorganisms. Specific parameters in gas fermentation change the type and concentration of the product produced (Lee et al., 2020; Yasin et al., 2019; Yasin et al., 2014). These numerous variables impact the optimization of the process system and the target chemical can be selected through optimization within the spectrum of strain growth conditions. The production of such chemicals can be enhanced or altered under the same reactor conditions, because the process operator modifies the target product in the same strain (Table 6). Eventually, the hurdles for successful C4 chemical production will not require existing facility reconfiguration or new factory construction but rather the optimization of major parameters and strain development. Acetic acid (C2 chemical) and butyric acid (C4 chemical) were used as examples. The unit price of acetic acid is 0.008 $ mol CO^{-1}, while that of butyric acid is 0.020 $ mol CO^{-1}, based on the theoretical maximum yield from CO. Although cost differences may arise depending on operating conditions, optimizing C4 chemicals to increase productivity when considering final profit is a more convenient commercialization approach, given that the final cost, which includes total variable and fixed costs, can be similar for both chemicals, and butyric acid reaches the break-even point sooner than acetic acid.

5. Research needs and future direction

Although syngas has potential as a next-generation biofuel, its applicability is still limited because syngas bioconversion mainly provides chemicals with low degree of reduction such as acetate and ethanol. Syngas fermentation-based biochemical production with a high degree of reduction offers considerable advantages in terms of bioprocess engineering, as existing facilities, including purification processes, can be applied without the need for infrastructure changes or new infrastructure. While C4 chemicals do not exhibit different properties compared to C2 chemicals, they may be produced under the same operating conditions using various types of bioreactors in gas fermentation and can be extracted using low-energy processes such as membrane separation (e.g., pervaporation) and electrochemical separation (e.g., electrodialysis). However, the major parameters controlling C4 chemical production need to be optimized, and further studies are required to achieve high-titer production. As reviewed in Sections 3 and 4, metabolic carbon flux and the resulting NADH/NAD^+ ratio appear to be the primary factors determining the majority of products. Energetics indicated that acetogens could maintain its viability and biocatalytic activities even if C4 chemicals were produced as a major product, provided sufficient substrates and reducing equivalents were supplied as needed. Since the efficiency of C4 chemical production was different depending on the metabolic characteristics of the strain, Group 1^b acetogens represented by *E. limosum* could be considered suitable strains for process operation despite *Clostridium*-based processes has been widely applied. In Section 4, some trials for syngas valorization previously used were covered, and direction of strain development and operation could be suggested based on the description. In the upstream phase, metabolic flux should be appropriately and correctly controlled. The flux control could be attempted in three ways. The first is the enhancement of the C4 chemical pathway, which is also presented in Section 4.1 and is the currently most common approach. Second, pathways for production of competitive chemicals can be inhibited. Although these approaches are "good" options for C4 chemical production, there is still a limitation to change C4 as major products due to redox balance of acetogens. If the rate of NADH generation required for C4 production is not sufficiently fast, energy yield of the strains could be easily unstable, affecting operation process. Therefore, flux control of enzymes related to redox

balance (such as Fdh, CODH and Hyd) must be also supported. The approach has not been tried, therefore, further research is needed. In the midstream phase, the simplest mixotrophy approach may be preferred. In the past, mixotrophy with sugar substrates (e.g., glucose and fructose) has been widely used. However, the substrates are not only costly but also follows the problem of edible biofuels. Methanol, which is cheap and abundant, can be used as a supplement that activate the NADH generation pathway of acetogens. Since methanol is known to be converted to CO_2 through methyl-THF in methylotrophic acetogens, NADH can be easily and quickly obtained. Therefore, methanol can be used as an excellent source of reducing equivalents for syngas valorization. The effect of methanol has been currently reported, it is thought that C4 chemicals might be produced as primary products when the mixotrophy approach is applied to genetically flux optimized strains through further research.

6. Conclusions

Economic feasibility of syngas bioconversion could be improved by applying upstream and midstream strategies to efficiently acquire reducing equivalents in biocatalysts. First, a suitable biocatalyst should be selected in consideration of metabolic features, especially the balancing type of reducing equivalents and energy. Second, the selected biocatalyst must be genetically developed toward direction for advantageously obtaining reducing equivalents. Finally, mixotropy approach could be applied as a way to gain reducing equivalents more easily. These approaches in the bioconversion will contribute to increasing TRLs of the process by making it possible to produce chemicals with high degree of reduction as main products.

CRediT authorship contribution statement

Ji-Yeon Kim: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Mungyu Lee:** Investigation, Writing – original draft. **Soyoung Oh:** Investigation. **Byeongchan Kang:** Investigation. **Muhammad Yasin:** Writing – review & editing. **In Seop Chang:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. As In Seop Chang, a [co-]author on this paper, is an editorial board member of Bioresource Technology, he was blinded to this paper during review, and the paper was independently handled by Samir Kumar Khanal as editor.

Data availability

Data will be made available on request.

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References

Abrini, J., Naveau, H., Nyns, E.-J., 1994. *Clostridium autoethanogenum*, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. Arch. Microbiol. 161 (4), 345–351.

Abubackar, H.N., Veiga, M.C., Kennes, C., 2011. Biological conversion of carbon monoxide: Rich syngas or waste gases to bioethanol. Biofuels Bioprod. Bioref. 5 (1), 93–114.

Alawan, H.A., Alminshid, A.H., Aljaafari, H.A.S., 2019. Promising evolution of biofuel generations. Subject review. Revew. Energy Focus. 28, 127–139.

Allen, T.D., Caldwell, M.E., Lawson, P.A., Huhnke, R.L., Tanner, R.S., 2010. *Alkalibaculum bacchi* gen. nov., sp. nov., a CO-oxidizing, ethanol-producing acetogen isolated from livestock-impacted soil. Int. J. Syst. Evol. Microbiol. 60 (10), 2483–2489.

Andreesen, J.R., Schaupp, A., Neurauter, C., Brown, A., Ljungdahl, L.G., 1973. Fermentation of glucose, fructose, and xylose by *Clostridium thermoaceticum*: effect of metals on growth yield, enzymes, and the synthesis of acetate from CO_2. J. Bacteriol. 114 (2), 743–751.

Arslan, K., Veiga, M.C., Kennes, C., 2021. Autotrophic (C1-gas) versus heterotrophic (fructose) accumulation of acetic acid and ethanol in *Clostridium aceticum*. Bioresour. Technol. 337, 125485.

Balk, M., Weijma, J., Friedrich, M.W., Stams, A.J., 2003. Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. Arch. Microbiol. 179 (5), 315–320.

Basen, M., Geiger, I., Henke, L., Müller, V., 2018. A genetic system for the thermophilic acetogenic bacterium *Thermoanaerobacter kivui*. Appl. Environ. Microbiol. 84 (3), e02210–e2307.

Baur, S.T., Markussen, S., Di Bartolomeo, F., Poehlein, A., Baker, A., Jenkinson, E.R., Daniel, R., Wentzel, A., Dürre, P., Nikel, P.I., 2022. Increased butyrate production in *Clostridium saccharoperbutylacetonicum* from lignocellulose-derived sugars. Appl. Environ. Microbiol. 88 (7), e0241921.

Bengelsdorf, F.R., Dürre, P., 2017. Gas fermentation for commodity chemicals and fuels. Microb. Biotechnol. 10 (5), 1167–1170.

Bengelsdorf, F.R., Poehlein, A., Linder, S., Erz, C., Hummel, T., Hoffmeister, S., Daniel, R., Dürre, P., 2016. Industrial acetogenic biocatalysts: a comparative metabolic and genomic analysis. Front. Microbiol. 7, 1036.

Bertsch, J., Müller, V., Lovell, C.R., 2015. CO metabolism in the acetogen *Acetobacterium woodii*. Appl. Environ. Microbiol. 81 (17), 5949–5956.

Bhatia, S.K., Kim, S.-H., Yoon, J.-J., Yang, Y.-H., 2017. Current status and strategies for second generation biofuel production using microbial systems. Energy. Convers. Manag. 148, 1142–1156.

Boga, H.I., Ludwig, W., Brune, A., 2003. *Sporomusa aerivorans* sp. nov., an oxygen-reducing homoacetogenic bacterium from the gut of a soil-feeding termite. Int. J. Syst. Evol. Microbiol. 53 (5), 1397–1404.

Bortolucci, J., Zani, A.C.B., de Gouvêa, P.F., Dinamarco, T.M., Reginatto, V., 2023. A non-solventogenic *Clostridium beijerinckii* strain lacking acetoacetate decarboxylase assimilates acetate and accumulates butyrate. Biomass. Bioenergy. 172, 106780.

Bosmans, A., Vanderreydt, I., Geysen, D., Helsen, L., 2013. The crucial role of Waste-to-Energy technologies in enhanced landfill mining: a technology review. J. Clean. Prod. 55, 10–23.

Braun, M., Mayer, F., Gottschalk, G., 1981. *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. Arch. Microbiol. 128 (3), 288–293.

Ceccaldi, P., Schuchmann, K., Müller, V., Elliott, S.J., 2017. The hydrogen dependent CO_2 reductase: the first completely CO tolerant FeFe-hydrogenase. Energy Environ. Sci. 10 (2), 503–508.

Cha, S., Lim, H.G., Kwon, S., Kim, D., Kang, C.W., Jung, G.Y., 2021. Design of mutualistic microbial consortia for stable conversion of carbon monoxide to value-added chemicals. Metab. Eng. 64, 146–153.

Chang, I.S., Kim, D.H., Kim, B.H., Shin, P.K., Yoon, J.H., Lee, J.S., Park, Y.-H., 1997. Isolation and identification of carbon monoxide utilizing anaerobe, *Eubacterium limosum* KIST612. Microbiol. Biotechnol. Lett. 25 (1), 1–8.

Chen, L., Msigwa, G., Yang, M., Osman, A.I., Fawzy, S., Rooney, D.W., Yap, P.-S., 2022. Strategies to achieve a carbon neutral society: a review. Environ. Chem. Lett. 20 (4), 2277–2310.

Cheng, C., Li, W., Lin, M., Yang, S.T., 2019. Metabolic engineering of *Clostridium carboxidivorans* for enhanced ethanol and butanol production from syngas and glucose. Bioresour. Technol. 284, 415–423.

Chew, K.W., Yap, J.Y., Show, P.L., Suan, N.H., Juan, J.C., Ling, T.C., Lee, D.-J., Chang, J.-S., 2017. Microalgae biorefinery: High value products perspectives. Bioresour. Technol. 229, 53–62.

Chew, K.W., Chia, S.R., Show, P.L., Ling, T.C., Arya, S.S., Chang, J.-S., 2018. Food waste compost as an organic nutrient source for the cultivation of *Chlorella vulgaris*. Bioresour. Technol. 267, 356–362.

Chowdhury, N.P., Litty, D., Müller, V., 2022. Biosynthesis of butyrate from methanol and carbon monoxide by recombinant *Acetobacterium woodii*. Int. Microbiol. 25 (3), 551–560.

Christensen, J.M., Jensen, P.A., Jensen, A.D., 2011. Effects of feed composition and feed impurities in the catalytic conversion of syngas to higher alcohols over alkali-promoted cobalt–molybdenum sulfide. Ind. Eng. Chem. Res. 50 (13), 7949–7963.

Daniell, J., Köpke, M., Simpson, S., 2012. Commercial biomass syngas fermentation. Energies 5 (12), 5372–5417.

Diekert, G., 1990. CO_2 reduction to acetate in anaerobic bacteria. FEMS Microbiol. Lett. 87 (3), 391–395.

Diekert, G.B., Thauer, R.K., 1978. Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. J. Bacteriol. 136 (2), 597–606.

Drake, H.L., Daniel, S.L., 2004. Physiology of the thermophilic acetogen *Moorella thermoacetica*. Res. Microbiol. 155 (10), 869–883.

Drake, H.L., Hu, S.I., Wood, H.G., 1980. Purification of carbon monoxide dehydrogenase, a nickel enzyme from *Clostridium thermocaceticum*. J. Biol. Chem. 255 (15), 7174–7180.

Drake, H.L., Gössner, A.S., Daniel, S.L., 2008. Old acetogens, new light. Ann. N. Y. Acad. Sci. 1125, 100–128.
Drake, H.L., 1994. Acetogenesis, acetogenic bacteria, and the acetyl-CoA "Wood/Ljungdahl" pathway: Past and current perspectives, in: Drake, H.L., Acetogenesis. Springer US. Boston, pp. 3-60.
Dürre, P., Sauer, M., 2016. Butanol formation from gaseous substrates. FEMS Microbiol. Lett. 363 (6), fnw040.
Extance, J., Crennell, S.J., Eley, K., Cripps, R., Hough, D.W., Danson, M.J., 2013. Structure of a bifunctional alcohol dehydrogenase involved in bioethanol generation in *Geobacillus thermoglucosidasius*. Acta Crystallogr. D Biol. Crystallogr. 69 (10), 2104–2115.
Fargione, J., Hill, J., Tilman, D., Polasky, S., Hawthorne, P., 2008. Land clearing and the biofuel carbon debt. Science 319 (5867), 1235–1238.
Fernández-Naveira, Á., Abubackar, H.N., Veiga, M.C., Kennes, C., 2016. Efficient butanol-ethanol (B-E) production from carbon monoxide fermentation by *Clostridium carboxidivorans*. Appl. Microbiol. Biotechnol. 100 (7), 3361–3370.
Fontaine, F.E., Peterson, W.H., McCoy, E., Johnson, M.J., Ritter, G.J., 1942. A new type of glucose fermentation by *Clostridium thermoaceticum*. J. Bacteriol. 43 (6), 701–715.
Gabris, C., Bengelsdorf, F.R., Dürre, P., 2015. Analysis of the key enzymes of butyric acid and acetic acid fermentation in biogas reactors. Microb. Biotehcnol. 8 (5), 865–873.
Gambelli, D., Alberti, F., Solfanelli, F., Vairo, D., Zanoli, R., 2017. Third generation algae biofuels in Italy by 2030: A scenario analysis using Bayesian networks. Energy Policy 103, 165–178.
Geerligs, G., Aldrich, H.C., Harder, W., Diekert, G., 1987. Isolation and characterization of a carbon monoxide utilizing strain of the acetogen *Peptostreptococcus productus*. Arch. Microbiol. 148 (4), 305–313.
Goodlove, P.E., Cunningham, P.R., Parker, J., Clark, D.P., 1989. Cloning and sequence analysis of the fermentative alcohol-dehydrogenase-encoding gene of *Escherichia coli*. Gene 85 (1), 209–214.
Gottschalk, G., Braun, M., 1981. NOTES: Revival of the name *Clostridium aceticum*. Int. J. Syst. Evol. Microbiol. 31 (4), 476.
Grethlein, A.J., Worden, R.M., Jain, M.K., Datta, R., 1991. Evidence for production of n-butanol from carbon monoxide by *Butyribacterium methylotrophicum*. J. Ferment. Bioeng. 72 (1), 58–60.
Hattori, S., Hongoh, Y., Itoh, T., Deevong, P., Trakulnaleamsai, S., Noparatnaraporn, N., Kudo, T., Ohkuma, M., 2013. *Sporomusa intestinalis* sp. nov., a homoacetogenic bacterium isolated from the gut of a higher termite, *Termes comis* (Termitinae). J. Gen. Appl. Microbiol. 59 (4), 321–324.
Hayes, C.J., Burgess, D.R., Manion, J.A., 2015. Chapter three – Combustion pathways of biofuel model compounds: A review of recent research and current challenges pertaining to first-, second-, and third-generation biofuels, in: Williams, I.H., Williams, N.H., Advances in Physical Organic Chemistry. Academic Press, pp. 103-187.
He, Y., Kennes, C., Lens, P.N.L., 2022. Enhanced solventogenesis in syngas bioconversion: Role of process parameters and thermodynamics. Chemosphere 299, 134425.
He, G., Lin, J., Sifuentes, F., Liu, X., Abhyankar, N., Phadke, A., 2020. Rapid cost decrease of renewables and storage accelerates the decarbonization of China's power system. Nat. Commun. 11 (1), 2486.
Heise, R., Reidlinger, J., Müller, V., Gottschalk, G., 1991. A sodium-stimulated ATP synthase in the acetogenic bacterium *Acetobacterium woodii*. FEBS. Lett. 295 (1–3), 119–122.
Heise, R., Müller, V., Gottschalk, G., 1993. Acetogenesis and ATP synthesis in *Acetobacterium woodii* are coupled via a transmembrane primary sodium ion gradient. FEMS Microbiol. Lett. 112 (3), 261–267.
Hermann, M., Popoff, M.-R., Sebald, M., 1987. *Sporomusa paucivorans* sp. nov., a methylotrophic bacterium that forms acetic acid from hydrogen and carbon dioxide. Int. J. Syst. Evol. Microbiol. 37 (2), 93–101.
Hess, V., Schuchmann, K., Müller, V., 2013. The ferredoxin:NAD^+ oxidoreductase (Rnf) from the acetogen *Acetobacterium woodii* requires Na^+ and is reversibly coupled to the membrane potential. J. Biol. Chem. 288 (44), 31496–31502.
Hess, V., Poehlein, A., Weghoff, M.C., Daniel, R., Müller, V., 2014. A genome-guided analysis of energy conservation in the thermophilic, cytochrome-free acetogenic bacterium *Thermoanaerobacter kivui*. BMC Genom. 15 (1), 1139.
Hess, V., Gallegos, R., Jones, J.A., Barquera, B., Malamy, M.H., Müller, V., 2016. Occurrence of ferredoxin:NAD^+ oxidoreductase activity and its ion specificity in several Gram-positive and Gram-negative bacteria. PeerJ. 4, e1515.
Huang, H., Chai, C., Li, N., Rowe, P., Minton, N.P., Yang, S., Jiang, W., Gu, Y., 2016. CRISPR/Cas9-based efficient genome editing in *Clostridium ljungdahlii*, an autotrophic gas-fermenting bacterium. ACS Synth. Biol. 5 (12), 1355–1361.
Jack, J., Lo, J., Maness, P.-C., Ren, Z.J., 2019. Directing *Clostridium ljungdahlii* fermentation products via hydrogen to carbon monoxide ratio in syngas. Biomass. Bioenergy. 124, 95–101.
Jeong, J., Kim, J.-Y., Park, B., Choi, I.-G., Chang, I.S., 2020. Genetic engineering system for syngas-utilizing acetogen, *Eubacterium limosum* KIST612. Bioresour. Technol. Rep. 11, 100452.
Jiang, B., Henstra, A.M., Paulo, P.L., Balk, M., van Doesburg, W., Stams, A.J., 2009. Atypical one-carbon metabolism of an acetogenic and hydrogenogenic *Moorella thermoacetica* strain. Arch. Microbiol. 191 (2), 123–131.
Joshi, S., Mishra, S., 2022. Recent advances in biofuel production through metabolic engineering. Bioresour. Technol. 352, 127037.
Kane, M.D., Breznak, J.A., 1991. *Acetonema longum* gen. nov. sp. nov., an H_2/CO_2 acetogenic bacterium from the termite, *Pterotermes occidentis*. Arch. Microbiol. 156 (2), 91–98.
Kang, H., Park, B., Bolo, N.R., Pathiraja, D., Park, S., Cha, M., Choi, I.-G., Chang, I.S., 2019. Gene-centric metagenome analysis reveals gene clusters for carbon monoxide conversion and validates isolation of a Clostridial acetogen for C2 chemical production. Biotechnol. J. 14 (6), e1800471.
Kanwal, S., Mehran, M.T., Hassan, M., Anwar, M., Naqvi, S.R., Khoja, A.H., 2022. An integrated future approach for the energy security of Pakistan: Replacement of fossil fuels with syngas for better environment and socio-economic development. Renewable Sustainable Energy Rev. 156, 111978.
Katsyv, A., Kumar, A., Saura, P., Pöverlein, C., Freibert, S.A., Stripp, S.T., Jain, S., Gamiz-Hernandez, A.P., Kaila, V.R.I., Müller, V., Schuller, J.M., 2023. Molecular basis of the electron bifurcation mechanism in the [FeFe]-hydrogenase complex HydABC. J. Am. Chem. Soc. 145 (10), 5696–5709.
Khoo, K.S., Lee, S.Y., Ooi, C.W., Fu, X., Miao, X., Ling, T.C., Show, P.L., 2019. Recent advances in biorefinery of astaxanthin from *Haematococcus pluvialis*. Bioresour. Technol. 288, 121606.
Khoo, K.S., Chew, K.W., Yew, G.Y., Leong, W.H., Chai, Y.H., Show, P.L., Chen, W.-H., 2020. Recent advances in downstream processing of microalgae lipid recovery for biofuel production. Bioresour. Technol. 304, 122996.
Kim, J.-Y., Park, S., Jeong, J., Lee, M., Kang, B., Jang, S.H., Jeon, J., Jang, N., Oh, S., Park, Z.-Y., Chang, I.S., 2021. Methanol supply speeds up synthesis gas fermentation by methylotrophic-acetogenic bacterium, *Eubacterium limosum* KIST612. Bioresour. Technol. 321, 124521.
Kita, A., Iwasaki, Y., Sakai, S., Okuto, S., Takaoka, K., Suzuki, T., Yano, S., Sawayama, S., Tajima, T., Kato, J., Nishio, N., Murakami, K., Nakashimada, Y., 2013. Development of genetic transformation and heterologous expression system in carboxydotrophic thermophilic acetogen *Moorella thermoacetica*. J. Biosci. Bioeng. 115 (4), 347–352.
Köpke, M., Held, C., Hujer, S., Liesegang, H., Wiezer, A., Wollherr, A., Ehrenreich, A., Liebl, W., Gottschalk, G., Dürre, P., 2010. *Clostridium ljungdahlii* represents a microbial production platform based on syngas. Proc. Natl. Acad. Sci. U.S.A. 107 (29), 13087–13092.
Köpke, M., Mihalcea, C., Liew, F., Tizard, J.H., Ali, M.S., Conolly, J.J., Al-Sinawi, B., Simpson, S.D., 2011. 2,3-butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. Appl. Environ. Microbiol. 77 (15), 5467–5475.
Kotsyurbenko, O.R., Simankova, M.V., Nozhevnikova, A.N., Zhilina, T.N., Bolotina, N.P., Lysenko, A.M., Osipov, G.A., 1995. New species of psychrophilic acetogens: *Acetobacterium bakii* sp. nov., *A. paludosum* sp. nov., *A. fimetarium* sp. nov. Arch. Microbiol. 163 (1), 29–34.
Kremp, F., Poehlein, A., Daniel, R., Müller, V., 2018. Methanol metabolism in the acetogenic bacterium *Acetobacterium woodii*. Environ. Microbiol. 20 (12), 4369–4384.
Kucek, L.A., Spirito, C.M., Angenent, L.T., 2016. High n-caprylate productivities and specificities from dilute ethanol and acetate: Chain elongation with microbiomes to upgrade products from syngas fermentation. Energy Environ. Sci. 9 (11), 3482–3494.
Kundiyana, D.K., Huhnke, R.L., Maddipati, P., Atiyeh, H.K., Wilkins, M.R., 2010. Feasibility of incorporating cotton seed extract in *Clostridium* strain P11 fermentation medium during synthesis gas fermentation. Bioresour. Technol. 101 (24), 9673–9680.
Lee, H., Bae, J., Jin, S., Kang, S., Cho, B.-K., 2022. Engineering acetogenic bacteria for efficient one-carbon utilization. Front. Microbiol. 13, 865168.
Lee, M., Yasin, M., Jang, N., Chang, I.S., 2020. A simultaneous gas feeding and cell-recycled reaction (SGCR) system to achieve biomass boosting and high acetate titer in microbial carbon monoxide fermentation. Bioresour. Technol. 298, 122549.
Leong, W.-H., Lim, J.-W., Lam, M.-K., Uemura, Y., Ho, Y.-C., 2018. Third generation biofuels: A nutritional perspective in enhancing microbial lipid production. Renew. Sustain. Energy Rev. 91, 950–961.
Li, F., Hinderberger, J., Seedorf, H., Zhang, J., Buckel, W., Thauer, R.K., 2008. Coupled ferredoxin and crotonyl coenzyme A (CoA) reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Etf complex from *Clostridium kluyveri*. J. Bacteriol. 190 (3), 843–850.
Liew, F., Henstra, A.M., Köpke, M., Winzer, K., Simpson, S.D., Minton, N.P., 2017. Metabolic engineering of *Clostridium autoethanogenum* for selective alcohol production. Metab. Eng. 40, 104–114.
Liou, J.S., Balkwill, D.L., Drake, G.R., Tanner, R.S., 2005. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. Int. J. Syst. Evol. Microbiol. 55 (5), 2085–2091.
Litty, D., Müller, V., 2021. Butyrate production in the acetogen *Eubacterium limosum* is dependent on the carbon and energy source. Microb. Biotechnol. 14 (6), 2686–2692.
Ljungdahl, L.G., 1994. The acetyl-CoA pathway and the chemiosmotic generation of ATP during acetogenesis, in: Drake, H.L., Acetogenesis. Springer US. Boston, pp. 63-87.
Luderer, G., Pehl, M., Arvesen, A., Gibon, T., Bodirsky, B.L., de Boer, H.S., Fricko, O., Hejazi, M., Humpenöder, F., Iyer, G., Mima, S., Mouratiadou, I., Pietzcker, R.C., Popp, A., van den Berg, M., van Vuuren, D., Hertwich, E.G., 2019. Environmental co-benefits and adverse side-effects of alternative power sector decarbonization strategies. Nat. Commun. 10 (1), 5229.
Lynd, L., Kerby, R., Zeikus, J.G., 1982. Carbon monoxide metabolism of the methylotrophic acidogen *Butyribacterium methylotrophicum*. J. Bacteriol. 149 (1), 255–263.
Maddipati, P., Atiyeh, H.K., Bellmer, D.D., Huhnke, R.L., 2011. Ethanol production from syngas by *Clostridium* strain P11 using corn steep liquor as a nutrient replacement to yeast extract. Bioresour. Technol. 102 (11), 6494–6501.
Marcellin, E., Behrendorff, J.B., Nagaraju, S., DeTissera, S., Segovia, S., Palfreyman, R. W., Daniell, J., Licona-Cassani, C., Quek, L.-E., Speight, R., Hodson, M.P., Simpson, S.D., Mitchell, W.P., Köpke, M., Nielsen, L.K., 2016. Low carbon fuels and commodity chemicals from waste gases – systematic approach to understand energy metabolism in a model acetogen. Green Chem. 18 (10), 3020–3028.

Mat Aron, N.S., Khoo, K.S., Chew, K.W., Show, P.L., Chen, W.-H., Nguyen, T.H.P., 2020. Sustainability of the four generations of biofuels – a review. Int J Energy Res 44 (12), 9266–9282.
Mechichi, T., Labat, M., Woo, T.H.S., Thomas, P., Garcia, J.-L., Patel, B.K.C., 1998. *Eubacterium aggregans* sp. nov., a new homoacetogenic bacterium from olive mill wastewater treatment digestor. Anaerobe 4 (6), 283–291.
Mock, J., Zheng, Y., Mueller, A.P., Ly, S., Tran, L., Segovia, S., Nagaraju, S., Köpke, M., Dürre, P., Thauer, R.K., Metcalf, W.W., 2015. Energy conservation associated with ethanol formation from H_2 and CO_2 in *Clostridium autoethanogenum* involving electron bifurcation. J. Bacteriol. 197 (18), 2965–2980.
Möller, B., Oßmer, R., Howard, B.H., Gottschalk, G., Hippe, H., 1984. *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov. Arch. Microbiol. 139 (4), 388–396.
Müller, V., 2003. Energy conservation in acetogenic bacteria. Appl. Environ. Microbiol. 69 (11), 6345–6353.
Munasinghe, P.C., Khanal, S.K., 2010. Biomass-derived syngas fermentation into biofuels: opportunities and challenges. Bioresour. Technol. 101 (13), 5013–5022.
Mutyala, S., Kim, J.R., 2022. Recent advances and challenges in the bioconversion of acetate to value-added chemicals. Bioresour. Technol. 364, 128064.
Nagaraju, S., Davies, N.K., Walker, D.J.F., Köpke, M., Simpson, S.D., 2016. Genome editing of *Clostridium autoethanogenum* using CRISPR/Cas9. Biotechnol. Biofuels 9 (1), 219.
Naik, S.N., Goud, V.V., Rout, P.K., Dalai, A.K., 2010. Production of first and second generation biofuels: a comprehensive review. Renew. Sustain. Energy Rev. 14 (2), 578–597.
Ollivier, B., Cordruwisch, R., Lombardo, A., Garcia, J.-L., 1985. Isolation and characterization of *Sporomusa acidovorans* sp. nov., a methylotrophic homoacetogenic bacterium. Arch. Microbiol. 142 (3), 307–310.
Pacheco, M., 2023. A systematic review of syngas bioconversion to value-added products from 2012 to 2022. Energies 16 (7), 3241.
Park, S., Yasin, M., Jeong, J., Cha, M., Kang, H., Jang, N., Choi, I.-G., Chang, I.S., 2017. Acetate-assisted increase of butyrate production by *Eubacterium limosum* KIST612 during carbon monoxide fermentation. Bioresour. Technol. 245, 560–566.
Peng, H., Wu, G., Shao, W., 2008. The aldehyde/alcohol dehydrogenase (AdhE) in relation to the ethanol formation in *Thermoanaerobacter ethanolicus* JW200. Anaerobe 14 (2), 125–127.
Philipps, G., de Vries, S., Jennewein, S., 2019. Development of a metabolic pathway transfer and genomic integration system for the syngas-fermenting bacterium *Clostridium ljungdahlii*. Biotechnol. Biofuels 12, 112.
Pierobon, S.C., Cheng, X., Graham, P.J., Nguyen, B., Karakolis, E.G., Sinton, D., 2018. Emerging microalgae technology: a review. Sustainable Energy Fuels. 2 (1), 13–38.
Poehlein, A., Schmidt, S., Kaster, A.-K., Goenrich, M., Vollmers, J., Thürmer, A., Bertsch, J., Schuchmann, K., Voigt, B., Hecker, M., Daniel, R., Thauer, R.K., Gottschalk, G., Müller, V., Driessen, A., 2012. An ancient pathway combining carbon dioxide fixation with the generation and utilization of a sodium ion gradient for ATP synthesis. PLOS ONE 7 (3), e33439.
Ragsdale, S.W., Pierce, E., 2008. Acetogenesis and the Wood-Ljungdahl pathway of CO_2 fixation. Biochim. Biophys. Acta – Proteins Proteom. 1784 (12), 1873–1898.
Rahayu, F., Kawai, Y., Iwasaki, Y., Yoshida, K., Kita, A., Tajima, T., Kato, J., Murakami, K., Hoshino, T., Nakashimada, Y., 2017. Thermophilic ethanol fermentation from lignocellulose hydrolysate by genetically engineered *Moorella thermoacetica*. Bioresour. Technol. 245, 1393–1399.
Rahbari, A., Ramdin, M., van den Broeke, L.J.P., Vlugt, T.J.H., 2018. Combined steam reforming of methane and formic acid to produce syngas with an adjustable H_2:CO ratio. Ind. Eng. Chem. Res. 57 (31), 10663–10674.
Richter, H., Molitor, B., Wei, H., Chen, W., Aristilde, L., Angenent, L.T., 2016. Ethanol production in syngas-fermenting *Clostridium ljungdahlii* is controlled by thermodynamics rather than by enzyme expression. Energy Environ. Sci. 9 (7), 2392–2399.
Schoelmerich, M.C., Katsyv, A., Sung, W., Mijic, V., Wiechmann, A., Kottenhahn, P., Baker, J., Minton, N.P., Müller, V., 2018. Regulation of lactate metabolism in the acetogenic bacterium *Acetobacterium woodii*. Environ. Microbiol. 20 (12), 4587–4595.
Schoelmerich, M.C., Katsyv, A., Dönig, J., Hackmann, T.J., Müller, V., 2019. Energy conservation involving 2 respiratory circuits. Proc. Acad. Sci. U. S. A. 117 (2), 1167–1173.
Schuchmann, K., Müller, V., 2013. Direct and reversible hydrogenation of CO_2 to formate by a bacterial carbon dioxide reductase. Science 342 (6164), 1382–1385.
Schuchmann, K., Müller, V., 2014. Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. Nat. Rev. Microbiol. 12 (12), 809–821.
Schulz, H., 1999. Short history and present trends of Fischer-Tropsch synthesis. Appl. Catal. A: Gen. 186 (1), 3–12.
Seifritz, C., Daniel, S.L., Gössner, A., Drake, H.L., 1993. Nitrate as a preferred electron sink for the acetogen *Clostridium thermoaceticum*. J. Bacteriol. 175 (24), 8008–8013.
Shah, S.H., Raja, I.A., Rizwan, M., Rashid, N., Mahmood, Q., Shah, F.A., Pervez, A., 2018. Potential of microalgal biodiesel production and its sustainability perspectives in Pakistan. Renew. Sustain. Energy Rev. 81, 76–92.
Sheldon, R.A., 2018. Enzymatic conversion of first- and second-generation sugars, in: Vaz Jr, S., Biomass and Green Chemistry: Building a Renewable Pathway. Springer International Publishing. Cham, pp. 169-189.
Shen, G.J., Annous, B.A., Lovitt, R.W., Jain, M.K., Zeikus, J.G., 1996. Biochemical route and control of butyrate synthesis in *Butyribacterium methylotrophicum*. Appl. Microbiol. Biotechnol. 45 (3), 355–362.
Shen, S., Gu, Y., Chai, C., Jiang, W., Zhuang, Y., Wang, Y., 2017. Enhanced alcohol titre and ratio in carbon monoxide-rich off-gas fermentation of *Clostridium carboxidivorans* through combination of trace metals optimization with variable-temperature cultivation. Bioresour. Technol. 239, 236–243.
Shin, J., Kang, S., Song, Y., Jin, S., Lee, J.S., Lee, J.K., Kim, D.R., Kim, S.C., Cho, S., Cho, B.K., 2019. Genome engineering of *Eubacterium limosum* using expanded genetic tools and the CRISPR-Cas9 system. ACS Synth. Biol. 8 (9), 2059–2068.
Shively, J.M., Barton, L., 1991. Variations in Autotrophic Life. Academic Press, London.
Simankova, M.V., Kotsyurbenko, O.R., Stackebrandt, E., Kostrikina, N.A., Lysenko, A.M., Osipov, G.A., Nozhevnikova, A.N., 2000. *Acetobacterium tundrae* sp. nov., a new psychrophilic acetogenic bacterium from tundra soil. Arch. Microbiol. 174 (6), 440–447.
Strätz, M., Gottschalk, G., Dürre, P., 1990. Transfer and expression of the tetracycline resistance transposon Tn925 in *Acetobacterium woodii*. FEMS Microbiol. Lett. 56 (1–2), 171–176.
Sun, X., Atiyeh, H.K., Huhnke, R.L., Tanner, R.S., 2019. Syngas fermentation process development for production of biofuels and chemicals: a review. Bioresour. Technol. Rep. 7, 100279.
Takors, R., Kopf, M., Mampel, J., Bluemke, W., Blombach, B., Eikmanns, B., Bengelsdorf, F.R., Weuster-Botz, D., Dürre, P., 2018. Using gas mixtures of CO, CO_2 and H_2 as microbial substrates: the do's and don'ts of successful technology transfer from laboratory to production scale. Microb. Biotechnol. 11 (4), 606–625.
Tanner, R.S., Miller, L.M., Yang, D., 1993. *Clostridium ljungdahlii* sp. nov., an acetogenic species in clostridial rRNA homology group I. Int. J. Syst. Evol. Microbiol. 43 (2), 232–236.
Traunecker, J., Preuß, A., Diekert, G., 1991. Isolation and characterization of a methyl chloride utilizing, strictly anaerobic bacterium. Arch. Microbiol. 156 (5), 416–421.
Tremblay, P.L., Zhang, T., Dar, S.A., Leang, C., Lovley, D.R., 2012. The Rnf complex of *Clostridium ljungdahlii* is a proton-translocating ferredoxin:NAD^+ oxidoreductase essential for autotrophic growth. mBio 4 (1) e00406-12.
Trischler, R., Roth, J., Sorbara, M.T., Schlegel, X., Müller, V., 2022. A functional Wood-Ljungdahl pathway devoid of a formate dehydrogenase in the gut acetogens *Blautia wexlerae*, *Blautia luti* and beyond. Environ. Microbiol. 24 (7), 3111–3123.
Ueki, T., Nevin, K.P., Woodard, T.L., Lovley, D.R., 2014. Converting carbon dioxide to butyrate with an engineered strain of *Clostridium ljungdahlii*. mBio 5 (5) e01636-14.
Vees, C.A., Herwig, C., Pflügl, S., 2022. Mixotrophic co-utilization of glucose and carbon monoxide boosts ethanol and butanol productivity of continuous *Clostridium carboxidivorans* cultures. Bioresour. Technol. 353, 127138.
Venkata Mohan, S., Lalit Babu, V., Sarma, P.N., 2008. Effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate. Bioresour. Technol. 99 (1), 59–67.
Wang, S., Huang, H., Moll, J., 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 (19), 5115–5123.
Wang, S., Huang, H., Kahnt, J., Mueller, A.P., Köpke, M., Thauer, R.K., 2013. NADP-specific electron-bifurcating [FeFe]-hydrogenase in a functional complex with formate dehydrogenase in *Clostridium autoethanogenum* grown on CO. J. Bacteriol. 195 (19), 4373–4386.
Wang, X., Qin, J., Ma, C., Wang, J., Wang, X., Xu, S., Feng, J., Chen, K., Ouyang, P., 2021. Methanol assimilation with CO_2 reduction in *Butyribacterium methylotrophicum* and development of genetic toolkits for its engineering. ACS Sustainable Chem. Eng. 9 (36), 12079–12090.
Wieringa, K.T., 1936. Over het verdwijnen van waterstof en koolzuur onder anaerobe voorwaarden. Antonie Leeuwenhoek. 3 (1), 263–273.
Wood, H.G., 1991. Life with CO or CO_2 and H_2 as a source of carbon and energy. FASEB J. 5 (2), 156–163.
Worden, R.M., Grethlein, A.J., Zeikus, J.G., Datta, R., 1989. Butyrate production from carbon monoxide by *Butyribacterium methylotrophicum*. Appl. Biochem. Biotechnol. 20 (1), 687–698.
Yasin, M., Park, S., Jeong, Y., Lee, E.Y., Lee, J., Chang, I.S., 2014. Effect of internal pressure and gas/liquid interface area on the CO mass transfer coefficient using hollow fibre membranes as a high mass transfer gas diffusing system for microbial syngas fermentation. Bioresour. Technol. 169, 637–643.
Yasin, M., Jang, N., Lee, M., Kang, H., Aslam, M., Bazmi, A.A., Chang, I.S., 2019. Bioreactors, gas delivery systems and supporting technologies for microbial synthesis gas conversion process. Bioresour. Technol. Rep. 7, 100207.
Zeikus, J.G., Lynd, L.H., Thompson, T.E., Krzycki, J.A., Weimer, P.J., Hegge, P.W., 1980. Isolation and characterization of a new, methylotrophic, acidogenic anaerobe, the Marburg strain. Curr. Microbiol. 3 (6), 381–386.