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MINI REVIEW

Production of biofuels from C_1-gases with *Clostridium* and related bacteria—Recent advances

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Abstract

Clostridium spp. are suitable for the bioconversion of C_1-gases (e.g., CO_2, CO and syngas) into different bioproducts. These products can be used as biofuels and are reviewed here, focusing on ethanol, butanol and hexanol, mainly. The production of higher alcohols (e.g., butanol and hexanol) has hardly been reviewed. Parameters affecting the optimization of the bioconversion process and bioreactor performance are addressed as well as the pathways involved in these bioconversions. New aspects, such as mixotrophy and sugar versus gas fermentation, are also reviewed. In addition, Clostridia can also produce higher alcohols from the integration of the Wood-Ljungdahl pathway and the reverse ß-oxidation pathway, which has also not yet been comprehensively reviewed. In the latter process, the acetogen uses the reducing power of CO/syngas to reduce C_4 or C_6 fatty acids, previously produced by a chain elongating microorganism (commonly *Clostridium kluyveri*), into the corresponding bioalcohol.

INTRODUCTION

Clostridia are anaerobic, endospore-forming bacteria that can metabolize a relatively broad range of substrates, including lignocellulosic, water-soluble and gaseous ones, resulting in the production of different possible metabolites, for example biofuels, platform chemicals and other valuable bio-based products. Some examples include *Clostridium phytofermentans* fermenting lignocellulosic biomass (Cerisy et al., 2017), *Clostridium acetobutylicum* metabolizing hexose and pentose sugars (Gomes et al., 2019) or *Clostridium aceticum* (Braun et al., 1981), *Clostridium autoethanogenum* (Abubackar et al., 2016) and *Clostridium ljungdahlii* (Phillips et al., 1993) growing on gases such as CO or CO_2. The most popular biofuel obtained from the bioconversion of soluble substrates (i.e. carbohydrates) by *Clostridium* spp. is butanol, resulting from the ABE (Acetone–Butanol–Ethanol) fermentation (Ezeji et al., 2004). With similar soluble substrates, the former process can be optimized in order to minimize the production of solvents and maximize hydrogen accumulation as an energy carrier (Kim et al., 1999). Biobutanol can be produced from different types of substrates, some of them, mainly wastes, are cost-effective, such as orange-peel waste (Su et al., 2022), food waste with any enzymatic pretreatment (Zhang et al., 2020) or starch (Qin et al., 2018).

This paper will mainly focus on straight C_1-gas (i.e. CO, CO_2/H_2 or syngas) fermentation to alcohols and recent advances related to aspects such as mixotrophy, as well as combined gas fermentation and chain elongation processes, yielding alcohols. This is also summarized in Table 1. Some C_1-gases are greenhouse

TABLE 1 Compilation of different strategies for obtaining alcohols by microorganisms involving *Clostridia*

	Process	Biofuel	Possible substrates	References
(a)	C_1-gas fermentation	Ethanol and/or higher alcohols (butanol and hexanol)	CO, CO_2/H_2, syngas, waste gases (e.g., steel industry emissions)	Fernández-Naveira et al. (2017a)
(b)	C_1-gas fermentation + sugar fermentation (mixotrophy)	Alcohols and/or higher alcohols (butanol and hexanol)	Glucose + gaseous electron donors (CO, CO_2/H_2)	Vees et al. (2022)
(c)	C_1-gas fermentation + chain elongation (co-cultivation)	Higher alcohols (butanol and eventually hexanol)	CO, CO_2/H_2, syngas, waste gases (e.g., steel industry emissions)	Richter et al. (2016)

Note: The different set-ups and reactor configurations of the processes summarized in Table 1 are illustrated in Figure 1.

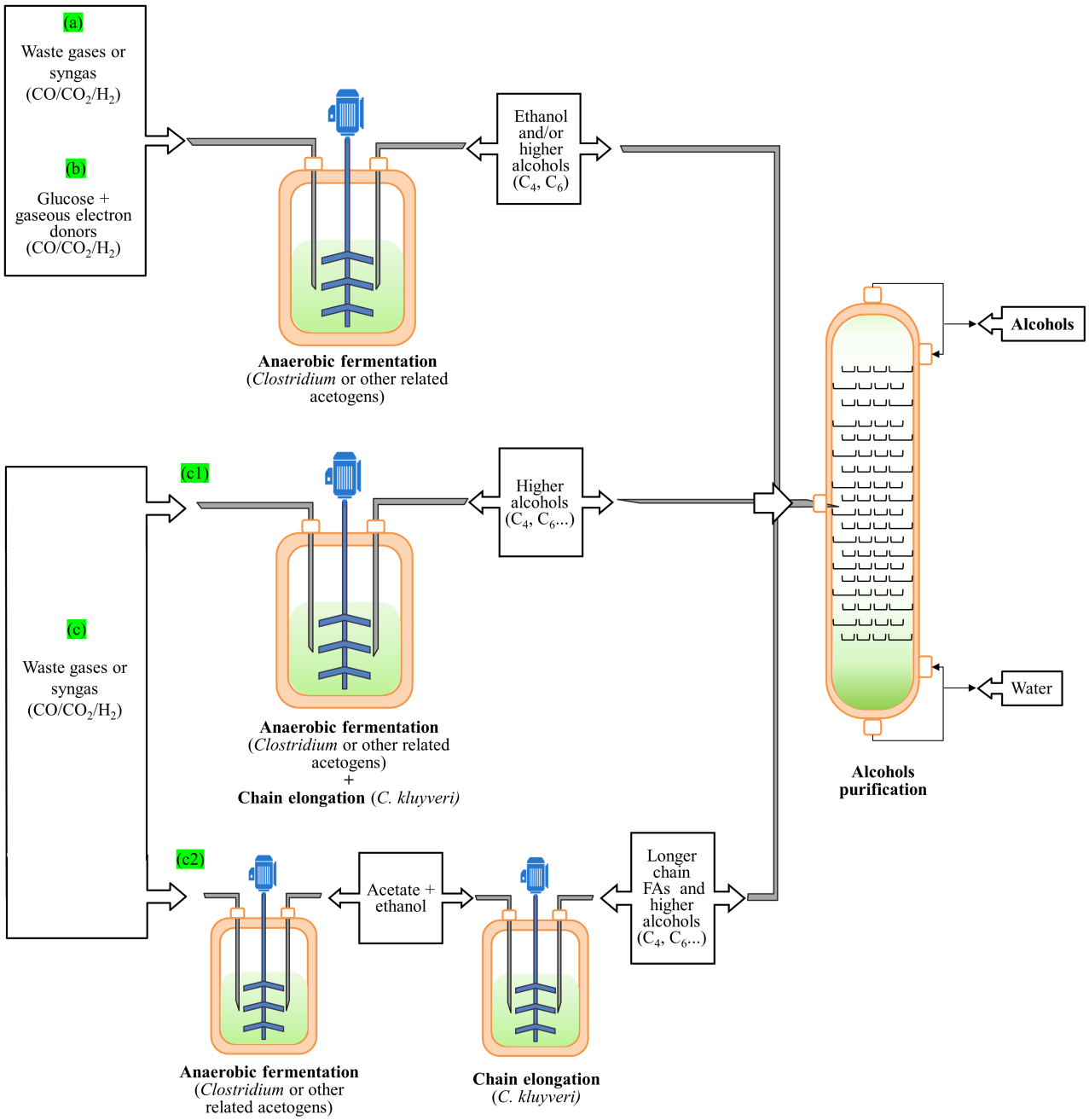

FIGURE 1 Different strategies for obtaining alcohols by microorganisms involving *Clostridia*. (a) Ethanol and higher alcohols production through C_1-gas (CO, CO_2, syngas) fermentation. (b) C_1-gas fermentation + sugar fermentation (mixotrophy). (c1) Integrated C_1-gas fermentation and chain elongation using co-culture strategy. (c2) Integrated C_1-gas fermentation and chain elongation using pure culture in two bioreactors.

gases (GHG), whose emission into the atmosphere and their consequences resulting from human activities are a real and concerning issue (Canatoy et al., 2022). Therefore, their capture and use to produce high value-added products represent not only a suitable environmental solution but also a way to avoid food-energy competition, when biofuels are obtained from agricultural products such as corn or sugarcane (Robles-Iglesias et al., 2023). In addition, another plus is that such gas fermentation has the potential to minimize waste gases from certain industries that use fossil fuels for generating power and heat. In the case of steelmaking processes, depending on the process, approximately 50% of the carbon used is emitted as CO (Bengelsdorf et al., 2018). Therefore, this is an innovative and promising technology that utilizes gaseous effluents containing CO and/or CO_2 and transforms them into commercial products such as sustainable fuels, while alleviating environmental issues such as global warming.

ETHANOL AND HIGHER ALCOHOLS PRODUCTION FROM C_1-GAS (CO, CO_2 AND SYNGAS) FERMENTATION

Ethanol and some other alcohols can either be produced through chemical catalytic processes or through bioprocesses (Kennes-Veiga et al., 2023). Today, a considerable amount of bioethanol is produced worldwide as a renewable fuel from the fermentation of carbohydrates available in maize, sugarcane or wheat, among others. Consequently, controversy has arisen due to competition between these crops for biofuels production or their use in the food industry, as in the ABE process. For the same reason, new technologies involving organic feedstocks of non-food origin are being developed. One of these alternatives is the thermopyrolysis of lignocellulosic materials to generate syngas composed of one-carbon compounds or C_1-gases (CO, CO_2) (Perez et al., 2013).

Syngas fermentation for acetate and ethanol production has experienced a growing interest in recent years due to its advantages over more traditional alternatives, such as its operation at low temperatures (e.g. 30–37°C) and atmospheric pressure (Arslan et al., 2019; Fernández-Naveira et al., 2019a; Mohammadi et al., 2012) and the high tolerance of biocatalysts to certain impurities (e.g. sulfur, chlorine) compared with chemical catalysts (Fernández-Naveira et al., 2017c). Besides, it is possible to produce ethanol and longer-chain alcohols such as butanol, hexanol and 2,3-butanediol from such gas fermentation as well (Köpke et al., 2011; Ramió-Pujol et al., 2015). This process can take place through three different mechanisms: (a) direct production of alcohols by acetogenic/solventogenic strains (Figure 1a), (b) mixotrophic fermentation of both gases and soluble substrates has recently also been addressed (Figure 1b), (c) indirect production combining syngas fermentation (performed by acetogenic bacteria) and chain elongation (commonly performed by species such as *C. kluyveri*) (Figure 1c). Concerning the first strategy, solvents can be produced by different acetogens from CO/CO_2/H_2 in various ratios and mixtures, including mainly *Clostridium carboxidivorans* (Fernández-Naveira et al., 2017a), *Clostridium aceticum* (Arslan et al., 2019), *Clostridium ljungdahlii* (Richter et al., 2016), *Clostridium ragsdalei* (Sun et al., 2018), *Clostridium autoethanogenum* (Liew et al., 2017), *Moorella thermoacetica* (Takemura et al., 2021), *Acetobacterium woodii* (Arslan et al., 2022), *Eubacterium limosum* (Litty & Müller, 2021) and *Thermoanaerobacter kivui* (Maru et al., 2018). Therefore, these species are currently of particular interest because they can use greenhouse gases (e.g. CO_2) as source of inorganic carbon and transform them into high added-value products. In this approach, ethanol is the most common alcohol, and the production of longer-chain alcohols (i.e. butanol and hexanol) is quite unusual. Besides *C. carboxidivorans*, there is hardly any other known microorganism so far that can efficiently produce such longer chain alcohols directly from syngas. A summary of some systems reported in the literature on the direct production of bioalcohols from syngas/CO/CO_2 by acetogens in pure and mixed cultures can be found in Table 2.

On the other hand, the primary end-products of acetogenic bacteria are acetate and, occasionally, ethanol, which are highly soluble in water and, therefore, their separation from the fermentation medium is energy-intensive, increasing the cost of the process significantly. Consequently, it is worth elongating these C_2 acids and alcohols, previously formed by acetogens, into longer-chain acids (C_4, C_6, C_8) in a cyclic process known as chain elongation performed by certain microorganisms, most notably *C. kluyveri* (Gildemyn et al., 2017). Although there are only few studies related to this, the appearance of long-chain alcohols (butanol, hexanol and sometimes even octanol) has been observed in this type of system where both technologies (i.e. gas fermentation and chain elongation) are merged (Diender et al., 2016; Richter et al., 2016). This fact is attributed to the reduction of the carboxylic acids present in the medium into their corresponding alcohols by the acetogen using the reducing power of CO and/or syngas. Finally, the production of alcohols can be enhanced in acetogens by combining the fermentation of sugars (e.g., glucose and fructose) and gases acting as elicitor agents (such as H_2, CO and syngas), in a process known as mixotrophy, combining autotrophic and heterotrophic feeding (Fast et al., 2015).

TABLE 2 Selection of different representative studies on the direct production of alcohols from C_1-gases with pure and mixed microbial cultures

Microorganism(s)	Substrate	Type of system	pH	Maximum alcohol concentrations reached (g/L)				References
				C_2	C_4	C_6	2,3-BD	
Pure cultures								
Clostridium carboxidivorans	Syngas	STBR	5.0	5.9	2.1	0.39	-	Fernández-Naveira et al. (2019b)[a]
Clostridium carboxidivorans	Syngas	STBR	5.75	2.7	1.9	0.85	-	Fernández-Naveira et al. (2017a)
Clostridium carboxidivorans	Syngas	Bottle study	~4.0–4.75	3.0	1.0	1.0	-	Phillips et al. (2015)
Clostridium carboxidivorans P7	CO (+ethanol)	Bottle study	5.0–6.0	2.19	2.55	8.45	-	Oh et al. (2023)[b]
Clostridium aceticum	Syngas	STBR	6.9	5.6	-	-	-	Arslan et al. (2021)
Clostridium aceticum	Syngas	STBR	6.6	3.2	-	-	-	Fernández-Blanco et al. (2022)
Clostridium ljungdahlii	Syngas	CSTR + cell recycle	4.5	48	-	-	-	Phillips et al. (1993)
Clostridium ljungdahlii	Syngas	CSTR	4	6.5	-	-	-	Mohammadi et al. (2012)
Clostridium ljungdahlii	Syngas/CO	CSTR	4.5	7.52	-	-	-	Younesi et al. (2006)
Clostridium ljungdahlii	Syngas	Bottle study	n.s.	1.0	-	-	0.15	Köpke et al. (2011)
Clostridium ragsdalei			n.s.	0.9	-	-	0.13	
Clostridium autoethanogenum			n.s.	1.0	-	-	0.18	
Clostridium autoethanogenum	CO	STBR	4.75 and 5.75 (shifts)	7.1	-	-	1.6	Abubackar et al. (2016)
Clostridium ragsdalei	Syngas	Bottle study	5.7–6.4	16.3	-	-	-	Sun et al. (2018)
Acetobacterium woodii	CO-formate	Bottle study	n.s.	0.18	-	-	-	Bertsch and Müller (2015)
Moorella thermoacetica	CO-H_2	Bottle study	6.9	0.14	-	-	-	Takemura et al. (2021)
Eubacterium limosum	CO/H_2-CO	Bottle study	6.0–7.7	s.a.	-	-	-	Litty and Müller (2021)
Eubacterium limosum KIST612	CO	BCTR	6.8	>0.09	-	-	-	Chang et al. (2001)
Mixed cultures								
Microbiome	CO_2/H_2	fermentation reactor	7	0.25	-	-	-	Xu et al. (2015)
Microbiome dominated by *Alkalibaculum bacchi* strain CP15 and *Clostridium propionicum*	Syngas	continuous ferm. + cell recycle	6.5–7.0	8.0	1.0	-	-	Liu et al. (2014)[c]
Microbiome	CO	Fed-batch	4.95–6.45	2.2	6.8	-	-	He et al. (2022)
Microbiome	CO	STBR	4.9	11.1	1.8	1.46	-	Chakraborty et al. (2019)

Abbreviations: 2,3-BD, 2,3-Butanediol; BCTR, bubble column reactor system; cell rec., cell recycle; CSTBR, continuous stirred tank bioreactor; n.s., not specified in the report; s.a., small amounts; STBR, stirred tank bioreactor.

[a]Maximum concentrations of C_2 and C_4 alcohols were reached in the same experiment, and C_6 alcohol concentration was reached in another experiment without W and Se.

[b]Maximum concentrations of C_4 and C_6 alcohols were reached in the same experiment (addition of ethanol and extractive oleyl alcohol), and C_2 alcohol concentration was reached in another experiment conditions (when using hexyl hexanoate as extracting solvent).

[c]The mixed culture produced 6 g/L of *n*-propanol as well. In this system, *A. bacchi* presumably reduced the *n*-propionate and the *n*-butyrate formed by *C. propionicum* to yield the corresponding alcohols.

ETHANOL PRODUCTION BY ACETOGENS FROM C_1-GASES

In autotrophic C_1-gas fermentation, acetogenic bacteria can produce ethanol as one of the most common end-metabolite, along with acetate, through the Wood-Ljungdahl (or acetyl-CoA) pathway (Figure 2a). Generally, ethanol production from CO takes place in two steps: (a) acetate synthesis in a process known as acetogenesis and (b) conversion of formerly produced acetate into ethanol takes place during solventogenesis (Arslan et al., 2019). Acetogenesis is favoured under optimal growth conditions for the bacteria, for example optimal pH, rich medium. Conversely, solventogenesis occurs under stress conditions or hindered cell growth (e.g. low pH, lack of nutrients) in the same way as in ABE fermentation (Al-Shorgani et al., 2018; Daniell et al., 2012; Fernández-Naveira et al., 2016b). Low-pH values are expected to trigger solventogenesis since the concentration of undissociated (protonated) acids, which are more toxic for microorganisms than the carboxylate non-dissociated (deprotonated) form, increases at the expense of the latter (Ganigué et al., 2016; Grethlein et al., 1990). It has been suggested that acetogens switch their metabolism from acetogenesis towards solventogenesis as a mechanism to avoid potential abrupt pH drops resulting from excessive acetate accumulation. This way, the bacteria would change from the exponential growth phase and acetogenesis to steady-state and then to solventogenesis and sporulation (Alsaker et al., 2010; Daniell et al., 2012).

The Wood-Ljungdahl metabolic pathway comprises an Eastern branch (methyl branch) and a Western branch (carbonyl branch), leading to the intermediate compound acetyl-CoA. The reducing power can come from organic and inorganic carbon sources (Katsyv & Müller, 2020), such as H_2 produced by hydrogenase enzymes and CO oxidation catalysed by carbon monoxide dehydrogenase (CODH) (Abubackar et al., 2011). Using this reductive pathway, ethanol production can be performed using CO and/or CO_2/H_2 as substrates according to Reactions 1 and 2:

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2 \quad (1)$$

$$6H_2 + 2CO_2 \rightarrow C_2H_5OH + 3H_2O \quad (2)$$

Given the stoichiometry of Reaction 1, it can be inferred that only 1/3 of the available CO is converted into ethanol since part of the CO is also used to form reducing equivalents after oxidation to CO_2.

If CO is used as the sole carbon source, one molecule of CO enters the Carbonyl branch, and another molecule is oxidized to CO_2 by CODH. The generated CO_2 then joins the Methyl branch to be reduced into a methyl group (methyl group of acetyl-CoA). On the other hand, if CO_2 alone is used as a carbon substrate, it will undergo several reducing steps in the Methyl branch to form a methyl group, while in the Carbonyl branch, CO_2 is reduced to CO via CODH/acetyl-CoA synthase (ACS). After several steps, the CODH/ACS complex catalyses the acetyl-CoA formation process

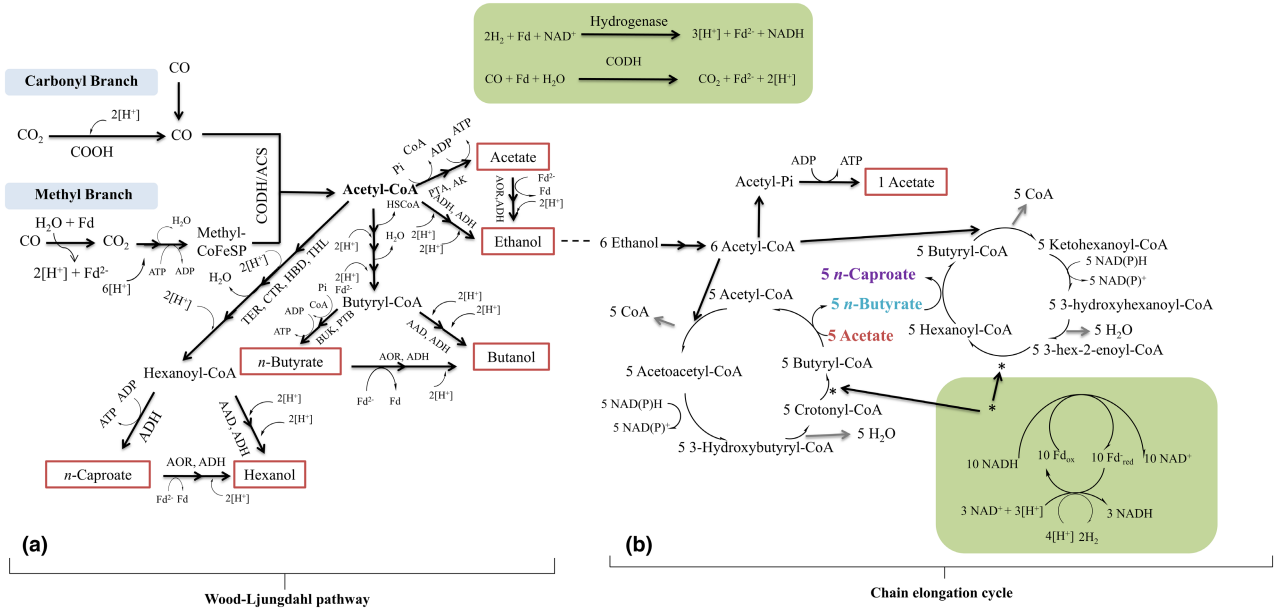

FIGURE 2 Hexanol–Butanol–Ethanol (HBE) pathway from C_1-gases. (a) Wood-Ljungdahl pathway (WLP) to produce volatile fatty acids and alcohols by acetogenic bacteria; (b) Reverse β-oxidation cycle used by chain elongation organisms; AAD, alcohol/aldehyde dehydrogenase; ADH, alcohol dehydrogenase; AK, acetate kinase; AOR, aldehyde: ferredoxin oxidoreductase; BUK, butyrate kinase; CODH/ACS, CO dehydrogenase/acetyl-CoA synthase; CTR, crotonase; Fd, oxidized ferredoxin; Fd^{-2}, reduced ferredoxin; HBD, 3-hydroxybutyryl-CoA dehydrogenase; PTA, phosphotransacetylase; PTB, phosphotransbutyrylase; TLH, thiolase.

by coupling the methyl and carbonyl groups and coenzyme A (CoA) (Bengelsdorf et al., 2013; Katsyv & Müller, 2020). Acetyl-CoA is the precursor for the formation of cell mass, acetate, and ATP during the cell growth phase, concomitant with acetogenesis and ethanol and NAD(P) production in the non-growth phase (during solventogenesis) (Abubackar et al., 2011). Likewise, it can be observed that the net synthesis of ATP by substrate phosphorylation in this pathway is zero, so additional cell energy production mechanisms for the cells must occur (Katsyv & Müller, 2020). Also, as shown in (Figure 2a), it is possible to produce several higher (bio)alcohols from acetyl-CoA (Fernández-Naveira et al., 2017c).

Many studies on ethanol production with acetogenic bacteria through the route mentioned above can be found in the literature. For example, Phillips et al. (1993) achieved the highest ethanol concentration reported in the scientific literature to date, which was 48 g/L after 560 h operation of a stirred tank bioreactor with cell recirculation and using a *C. ljungdahlii* strain as biocatalyst and grown in a designed fermentation medium without yeast extract. The maximum cell concentration was 4 g/L, whereas CO bioconversion was around 90% during most of the experiment. These same authors suggested that a pH range of 4.0–4.5, high mass transfer from an adequate supply of syngas, and minimal medium are conditions that favour ethanol production in this microorganism. Using that same species, Younesi et al. (2006) showed that high amounts of ethanol (up to about 7 g/L) could be produced, together with acetic acid, from syngas and pure CO in a continuous process for more than 3 months, reaching a maximum cell concentration and CO bioconversion of 2.1 g/L and 93%, respectively. Likewise, in a fermentation with a pure culture of *C. ljungdahlii* at pH lower than 5, ethanol productivity was higher when using syngas (60% CO, 35% H_2, and 5% CO_2) as substrate instead of CO. Specifically, ethanol concentration ranged from 0.30 ± 0.07 to 1.3 ± 0.05 g/L when using CO as substrate, compared with ethanol variation from 0.80 ± 0.02 to 3.75 ± 0.08 g/L during syngas fermentation (Acharya et al., 2019).

On the other hand, a culture medium containing red cedar or poultry litter improved ethanol production in *C. ragsdalei* by 16.3% and 58.9%, respectively, compared to a medium containing yeast extract (Sun et al., 2018). In the case of the medium rich in poultry litter with an inoculum 138 h aged; the final ethanol concentration was 16.25 g/L with a CO utilization level of 54.21%. Another study showed that *C. carboxidivorans* has a higher potential for solvent production from CO_2 and H_2 than the acetogen *C. ragsdalei*, reaching higher ethanol-to-acetate ratios and higher ethanol yields, producing 33% more ethanol and 66% less acetate. In addition, a medium rich in cotton seed extract contributes to ethanol production in both strains (Ramachandriya et al., 2013). Also, it was shown how a strategy based on cyclic pH shifts of high (5.75) and low (4.75) pH values is a feasible approach for reaching high concentrations of alcohols, up to 7.1 g/L of ethanol with no accumulation of acetate, with *C. autoethanogenum* from CO-rich gases (Abubackar et al., 2016). Another acetogenic bacterium that deserves to be mentioned is *C. aceticum*. It has the singularity that, in this organism, the solventogenesis phase occurs at pH values close to neutrality, as opposed to the slightly acidic conditions required by all other bacteria listed in Table 1. For example, Arslan et al. (2019) achieved a maximum concentration of 5.6 g/L ethanol at pH 6.9 after allowing the medium to acidify naturally, from the initially slightly alkaline conditions, through the accumulation of acetate formed from a syngas mixture in an STBR. In a rather similar system, Fernández-Blanco et al. (2022) observed that the ethanol production rate after acetate accumulation from syngas fermentation was highest at pH 6.6 with a pure culture of *C. aceticum*. However, ethanol production from C_1-gases is feasible with pure and mixed cultures, which is especially interesting when a mixture of different acids or other metabolites is desired. For instance, a culture of *Alkalibaculum bacchi* strain CP15 (56%), which was found to be contaminated with *Clostridium propionicum* (34%), used syngas to yield maximum ethanol, *n*-propanol, and *n*-butanol concentrations of 8, 6 and 1 g/L, respectively, in a 7 L fermenter with a medium containing 20 g/L corn steep liquor and with cell recycle (Liu et al., 2014).

Thus, the production of bioethanol from C_1-gases is a viable and promising process that represents an alternative for producing more environmentally friendly fuels than petroleum derivatives ones, also resulting in lower emissions of greenhouse gases and even consumption of the latter (Kennes et al., 2016). This alternative has even very recently reached commercial scale with the first gas fermentation plant producing ethanol and set up by the company Lanzatech after about a decade of fruitful research and optimization activities (Köpke & Simpson, 2020). Nevertheless, the technology still deserves further optimization, especially in maximizing productivity without compromising bacterial activity due to excessive accumulation of undissociated acids, low-pH levels and other stressful conditions for the cells.

PARAMETERS FAVOURING ALCOHOLS VS. ACIDS PRODUCTION

The primary product of acetogens under optimal growth conditions is acetate; however, increasing the ethanol/acetate ratio is desirable to stimulate alcohol-biofuels' production, or even manage to produce ethanol as

single end-product. Therefore, in recent years, research has been undertaken to elucidate the most important factors that enhance ethanol production at the expense of acetate.

As discussed in the previous section, solventogenesis, or the production of alcohols, would be enhanced over acetogenesis at pH values below the optimum pH for growth of solventogenic bacteria, usually by more than 1 pH unit. Typically, a high pH period is needed first to favour both bacterial growth and acetogenesis; then, after the exponential growth phase and once a given acetate concentration has been reached, the pH can be lowered either artificially or naturally (as a result of the accumulation of acetate itself) to trigger its bioconversion into ethanol (Abubackar et al., 2016; Arslan et al., 2019; Fernández-Naveira et al., 2019b; Mohammadi et al., 2012). Figure 3 plots the different common optimal pH values for acidogenesis and solventogenesis for different *Clostridium* strains grown on C_1-gases under specific experimental conditions (Abubackar et al., 2015; Arslan et al., 2019; Fernández-Naveira et al., 2017a; Kundiyana et al., 2011; Mohammadi et al., 2012). In the case of *C. ragsdalei*, the pH for solventogenesis shown in the figure is the final pH of fermentation, since the exact pH value at which the acidogenesis–solventogenesis phase shift occurs was not mentioned. Also, the condition under which the maximum ethanol concentration was reached has been plotted.

Besides, specific trace metals also play an essential role in steering the metabolism of C_1-gas metabolizing bacteria towards either acetogenesis or solventogenesis. Specifically, recent studies have reported that the presence of tungsten (W) in the fermentation medium has a significant positive effect on the production of alcohols (e.g. ethanol) compared to bioconversion in its absence, both in pure and mixed cultures. Other trace metals, such as selenium (Se), also seem to contribute to solventogenesis, although their effect is quite less noticeable and sometimes contradictory, depending on the strain and operating conditions, among others (Abubackar et al., 2015; Chakraborty et al., 2020; Fernández-Naveira et al., 2019b). Likewise, a reduced molybdate (Mo) dosage has been reported to improve alcohol production as it appeared to promote carbon fixation and solventogenesis in *C. carboxidivorans* P7 (Han et al., 2020). In *C. carboxidivorans* P7, alcohol productivity was also improved in the presence of excess Zn^{2+}, resulting in a 3.02-fold enhancement of ethanol content, respectively, through an increase in the gene expression of carbon fixation and alcohol dehydrogenase, thus regulating the *de novo* synthesis of alcohols (Li et al., 2018).

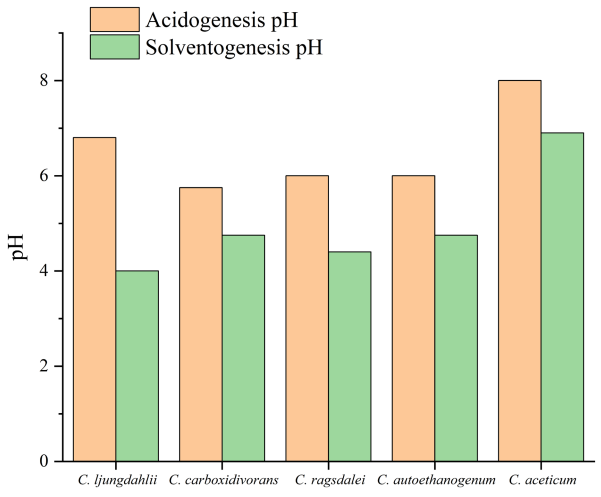

FIGURE 3 Acidogenic and solventogenic pH values for different *Clostridium* spp. under specific conditions during C_1-gas fermentation.

Another parameter that can affect the production of ethanol, and other alcohols, is the incubation temperature. A study with *C. carboxidivorans* P7 revealed that temperatures below the optimum acetogenic growth temperature led to higher productivities of alcohols, thus overcoming the 'acid crash' phenomenon described in a former section. In the case of ethanol, a concentration of 1.48 g/L was achieved at 25°C compared with 0.072 g/L at the optimum temperature of 37°C (Ramió-Pujol et al., 2015). These results agree with the study reported by Shen et al. (2017), who optimized the trace metals composition of their medium and worked with time-controlled temperature (37°C during the first 24 h and then 25°C). This temperature strategy and trace metal makeup increased the concentration of alcohols up to 6.97 g/L.

Finally, concerning the nitrogen source, Gaddy and Clausen (1992) demonstrated that when *C. ljungdahlii* was subjected to non-growth conditions (i.e. pH 4.0 and yeast extract fully removed), the ethanol concentration exceeded that of acetate. Thus, at pH 5.0 and in batch mode, the ethanol/acetate molar ratio achieved was approximately 0.11 in the presence of small quantities of yeast extract (0.005%, 0.01%, 0.05%), in contrast to a ratio of 0.05 at higher concentrations of yeast extract (0.1%, 0.2%). Likewise, the substitution of yeast extract for steep corn liquor also led to a 78% improvement in ethanol productivity in a mixed syngas-fed culture (Liu et al., 2014). According to the authors, the reason might be because micronutrients in steep corn liquor media are present in higher amounts or are not present at all in YE (yeast extract) medium.

It can be observed from the literature that several studies have been focussing on maximizing the production of biofuels (e.g. bioethanol) through the modification of experimental parameters. However, further research is still needed on different operating conditions to improve the ethanol/acetate ratio in the culture broth, such as, for example the implementation of biomass recirculation (Phillips et al., 1993), a two-stage continuous fermentation (Gaddy & Clausen, 1992) or even metabolic engineering (Harris et al., 2000).

BUTANOL AND HEXANOL PRODUCTION, BESIDES ETHANOL, BY ACETOGENS FROM C_1-GASES

The term HBE (Hexanol-Butanol-Ethanol) fermentation was coined by Fernández-Naveira et al. (2017b) to refer to the production of such mixture of bioalcohols from C_1-gases. This acronym is derived from the ABE fermentation mentioned above.

The metabolic pathway from which these metabolites are formed is, here also, the Wood-Ljungdahl pathway (Figure 2a). As for *n*-butanol, acetyl-CoA is first enzymatically converted to butyryl-CoA, which can be a precursor for the direct formation of *n*-butyrate and *n*-butanol. *n*-Butyrate formed via butyryl-CoA can also be converted to butyraldehyde, catalysed by ferredoxin:aldehydeoxydoreductase (AOR), and subsequently reduced to yield *n*-butanol. Regarding *n*-hexanol, acetyl-CoA is first transformed into hexanoyl-CoA as explained in Fernández-Naveira et al. (2017c), which can directly yield either *n*-caproate (i.e., *n*-hexanoate) or *n*-hexanol, producing hexaldehyde as an intermediate compound.

So far, the only known bacterial species capable of efficiently performing this bioconversion to produce higher alcohols from C_1-gases is *C. carboxidivorans*. In this organism, the procedure for stimulation of solventogenesis is similar as in other solventogenic bacteria producing ethanol only. First, high pH levels (i.e., around 6.0) are required for optimal cell growth. Once given concentrations of acetate, *n*-butyrate, and *n*-caproate have been reached, the pH can be lowered (i.e., 4.8–5.0), favouring the bioconversion of the acids into their respective bioalcohols (Fernández-Naveira et al., 2019a). Actually, under continuous gas feed and liquid batch conditions, initially maintaining an optimal pH for growth allows the accumulation of higher concentrations of acids than when the pH is not controlled, resulting thus in faster, natural acidification before high amounts of acids can accumulate. Therefore, higher concentrations of alcohols can be obtained after the reduction in high amounts of accumulated acids (Fernández-Naveira et al., 2017a). At pH 5.75, in an STBR with a continuous syngas supply, the highest concentrations of ethanol, *n*-butanol and *n*-hexanol were, respectively, 2.7, 1.9 and 0.85 g/L. At the same time, the best production rates of these bioalcohols were found at pH 4.75, that is 0.048 g ethanol h^{-1} g^{-1} biomass, 0.037 g butanol h^{-1} g^{-1} biomass and 0.026 g hexanol h^{-1} g^{-1} biomass. Also, 10 times higher concentrations of ethanol and *n*-butanol were obtained from the fermentation of syngas in a molybdate-deficient medium compared with the use of the original trace metals, reaching values of 2.0 and 1.0 g/L, respectively, together with 0.5 g/L *n*-hexanol at the end of the experiment (Han et al., 2020). In another study, Phillips et al. (2015) successfully grew a strain of *C. carboxidivorans* in a designed medium to enhance the production of *n*-butanol and *n*-hexanol. The medium lacked yeast extract, 2-(N-morpholino)ethanesulfonic acid, and minimal complex chemical inputs. An initial CO partial pressure of 30 kPa was used, without agitation, with a programmed increase in CO partial pressure up to 90 kPa and increased agitation up to 125 rpm and then up to 150 rpm, thus reducing substrate inhibition and mass transfer limitation. With this cultivation technique, it was possible to increase the production of *n*-butanol and *n*-hexanol, reaching values higher than 1.0 and 1.0 g/L, respectively, in-bottle fermentation. Under the previously mentioned conditions of temperature change (high-to-low) and optimized trace metals composition, Shen et al. (2017) achieved concentrations of 1.67 g/L *n*-butanol and 1.33 g/L *n*-hexanol from artificial steel mill off-gas fermentation with *C. carboxidivorans* P7. Besides, a recent study demonstrated that extractive fermentation of CO with a pure culture of *C. carboxidivorans* P7 significantly improves hexanol production. Specifically, oleyl alcohol was selected as the extractant agent, which was repeatedly added during fermentation, achieving values of up to 5.06 g/L hexanol. Hexanol productivity still increased to 8.45 g/L when not only oleyl alcohol but also ethanol was repeatedly added during fermentation (Oh et al., 2023).

Lastly, since this bacterium produces many products with a longer hydrocarbon chain, one of the major concerns is the potential toxicity exerted by these compounds on the microorganism. Fernández-Naveira et al. (2016a) conducted a toxicity study with *C. carboxidivorans*. The results indicated that *n*-butanol has a higher inhibitory effect than ethanol on cells and a lower IC_{50} (i.e., concentration leading to 50% inhibition). Concentrations of 14–14.50 g/L *n*-butanol caused 50% growth inhibition, while a concentration of 20 g/L appeared to be lethal to *C. carboxidivorans*. However, 35 g/L ethanol is necessary to decrease the concentration of the cells by 50%, suggesting that this alcohol is relatively less inhibitory to acetogenic bacteria than higher alcohols.

Furthermore, besides pure cultures, mixed cultures have also been studied. In that sense, a mixed culture of solventogenic bacteria was enriched from anaerobic granular sludge of dairy wastewater, thermally treated at pH 5.7–6.5, and inoculated in a bioreactor with intermittent CO feed to produce high concentrations of alcohols under stress conditions (i.e., low pH and initial pressure of 1.8 bar CO), such as ethanol (2.2 g/L) and butanol (6.8 g/L) (He et al., 2022). This is the highest butanol concentration ever reported from C_1-gas fermentation, as pure culture studies do, so far, generally not exceed 2 g/L butanol. Similarly, Chakraborty et al. (2019) achieved maximum ethanol, butanol and hexanol concentrations of, respectively, 11.1, 1.8 and 1.46 g/L after acclimating an anaerobic granular sludge to CO and operating the bioreactor under conditions

that foster solventogenesis, which consisted in applying first a high pH value of 6.2 to produce fatty acids with their subsequent bioconversion to alcohols at low pH 4.9.

C_1-GAS FERMENTATION + SUGAR FERMENTATION (MIXOTROPHY)

One of the significant disadvantages of C_1-gas/syngas fermentation to produce either VFAs (volatile fatty acids) or alcohols is the low solubility of these gases in water, which inevitably limits productivity. Moreover, the Wood-Ljungdahl route does not produce the net ATP required for efficient biosynthesis by substrate-level phosphorylation (Fast et al., 2015). One way to compensate for this ATP limitation could be mixotrophy or combining organic sugars (heterotrophy) and C_1-gas (autotrophy) fermentation (Figure 1b). This way, the ATP generated from glycolysis can be used for cell growth. The CO_2 released during this process can be assimilated by the acetogens as shown in Figure 4, thus improving the net fermentation yield (He et al., 2022). This would also help address the lack of energy in sugar fermentation, resulting in the impossibility of achieving complete carbon fixation (Maru et al., 2018). In addition to the CO_2 liberated from heterotrophy, the possibility of feeding gaseous electron donors (i.e., H_2, CO) from pure or mixed (syngas) gases to stimulate mixotrophy in acetogens has recently been studied. For example, supplemental CO feeding in a sugar-rich system could increase the available acetyl-CoA pool to perform chain elongation and simultaneously partially oxidize to CO_2 to supply Fd^{2-}, necessary for reducing the carboxylates to their respective alcohols (Vees et al., 2022).

Most acetogenic *Clostridia* can grow under mixotrophic conditions, and the cultivation in the presence of both carbohydrates and C_1-gases can have some advantages. However, research has shown that different minor metabolites may also be produced. In a recent study in a continuous system with *C. carboxidivorans* in the presence of 10 g/L glucose and 20% CO, ethanol and butanol production were improved 1.7 and 1.5 times, respectively, concerning the heterotrophic conditions (10 g/L glucose). However, the alcohol: acid ratio did not change. Overall, the maximum concentrations of alcohols achieved were 5.7 g/L ethanol, 2.6 g/L butanol and 0.7 g/L hexanol, and the combined alcohol titre was 9.1 g/L, which represents an improvement of 148% compared to heterotrophy. On the other hand, mixotrophy with syngas ($CO+CO_2+H_2$) and glucose increased the combined titration of alcohols by a factor of 1.2 and ethanol production by 80% compared with heterotrophic conditions. Also, a 2.2-fold higher rate of specific CO_2 evolution was observed compared with heterotrophy, reflecting that CO is partially oxidized to CO_2 to serve as a source of electrons, from the generation of reduced ferredoxin, and only partially as a carbon source. Also, H_2 feeding did not seem to affect metabolite, CO_2, and biomass production, as there was no appreciable H_2 uptake (Vees et al., 2022). Overall, CO mixotrophy showed the best results in terms of metabolite titers with final concentrations of ethanol, butanol and hexanol of 5.7, 2.6 and 0.7 g/L.

In another study with *C. carboxidivorans*, it has been shown that the metabolite production profile is somewhat different when only glucose is used as substrate versus C_1-gas fermentation. Under these conditions, the strain produced a wider range of metabolites, such as acetate, butyrate, caproate, formate and lactate. Conversely, the latter two are not common end metabolites in gas fermentation and are hardly ever detected (Fernández-Naveira et al., 2017b). Lactate and formate appeared at a high pH of 6.2 and were then converted into acetate. Nevertheless, after applying the typical pH strategy of decreasing pH to stimulate solventogenesis, previously discussed, metabolizing the acids to give their respective alcohols, as is usual in C_1-gas fermentation, was not observed. Only minor concentrations of alcohols were obtained at higher pH values and not under more acidic conditions.

A similar behaviour was reported by Arslan et al. (2021) in another organism, that is a *C. aceticum* strain grown on fructose, in which acidification of the

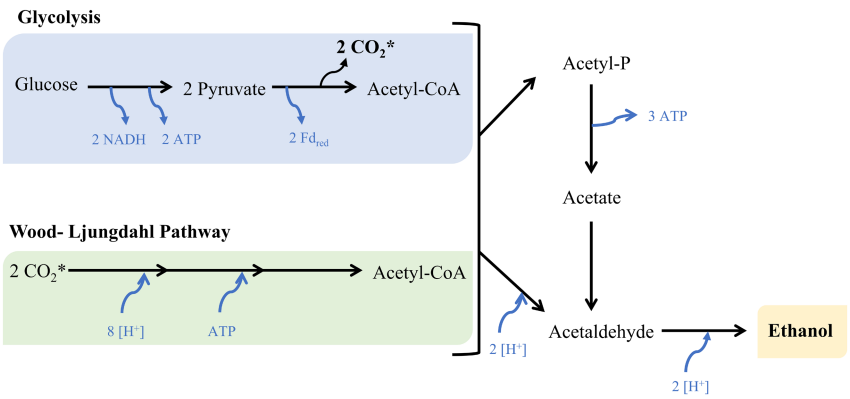

FIGURE 4 Combined glycolysis and Wood-Ljungdahl (simplified) pathways in acetogenic bacteria for ethanol production.

medium did not stimulate the production of alcohols in this case either. In contrast, it did when CO was used as the only substrate. In this study, the accumulation of formate was also observed, which ended up exerting a robust inhibitory effect on the microorganism at the optimum pH of 8.

Thus, it can be concluded that the productivity profile and the spectrum of final metabolites in acetogens depend on the carbon source. Mixotrophy is an approach with the potential to make up for the energetic or carbon loss limitations that autotrophy and heterotrophy have separately.

INTEGRATED C_1-GAS FERMENTATION AND CHAIN ELONGATION THROUGH REVERSE ß-OXIDATION

Another recently reported method yielding higher bioalcohols from C_1-gases consists in combining syngas fermentation and chain elongation (Figure 1c1,c2). This way, acetate and ethanol produced by acetogenic bacteria from C_1-gases act as electron donors and electron acceptors, respectively, to elongate their chain and produce longer, medium-chain, fatty acids, that is *n*-butyrate, *n*-caproate and even *n*-caprylate. Chain-elongating bacteria, such as *C. kluyveri* (the most widely studied chain-elongating microorganism), carry out this cyclic pathway based on reverse β-oxidation. In this route, first, part of the ethanol is oxidized to acetate via substrate phosphorylation, generating energy in the form of ATP. The remaining ethanol is transformed into acetyl-CoA (Figure 2b). Next, the acetyl-CoA molecule binds to acetate, elongating its chain by two carbons and generating *n*-butyrate.

In the same way, *n*-butyrate is transformed into *n*-caproate after adding another molecule of acetyl-CoA (Angenent et al., 2016; Cavalcante et al., 2017; Spirito et al., 2014). This combination of two different fermentation processes is a suitable alternative to obtain higher added-value, longer chain products with a higher market price than C_2 compounds (e.g., acetic acid). However, it is common in this kind of system to detect longer than two-carbon bioalcohols besides medium-chain C_4-C_8 fatty acids, as shown in Table 3. This is because acetogenic bacteria seem to have the ability to reduce the accumulated fatty acids into their respective alcohols, using this syngas/CO as an electron (reductive power) and energy source (Perez et al., 2013). Years earlier, it had been shown that carboxylic acids could be reduced into their respective alcohols using hydrogen as the sole electron donor in the presence of granular sludge (Steinbusch et al., 2008).

Co-cultures with two single species, that is an acetogen and *C. kluyveri*, performing syngas fermentation and chain elongation, respectively, can be tailored

TABLE 3 Different studies on the indirect production of alcohols, combining syngas fermentation and chain elongation, from C_1-gases with pure and mixed cultures

Microorganism(s)	Substrate	Type of system	pH	Maximum alcohol concentration reached (g/L)			References
				C_4	C_6	C_8	
Co-cultures (two bacterial species)							
C. autoethanogenum + *C. kluyveri*	CO/syngas	Bottle study	6.0–7.2	0.43±0.1	0.43±0.2	-	Diender et al. (2016)
C. ljungdahlii + *C. kluyveri*	Syngas	continuous syst. + in-line product extrac.	5.7–6.4	4.2	4.7	0.78	Richter et al. (2016)
C. carboxidivorans + *C. kluyveri*	Syngas	Batch-STBR	6.0	0.84±0.06	0.24±0.01	-	Bäumler et al. (2021)
C. aceticum + *C. kluyveri*	Syngas + ethanol	STBR	6.6–7.5	0.72	-	-	Fernández-Blanco et al. (2022)
Mixed culture							
Microbiome	Syngas	STBR	4.8	1.1	0.6	-	Ganigué et al. (2016)

Abbreviations: extrac., extraction; STBR, stirred tank bioreactor; subs., substrate; syst., system.

to promote beneficial interactions between those microorganisms while preventing unwanted side reactions, more typical in open mixed cultures, that would negatively affect the process performance (Diender et al., 2021). For example, *C. kluyveri* co-cultured with *C. carboxidivorans* (Figure 1c1) generated a threefold increase in the final *n*-butanol concentration and allowed the production of *n*-hexanol when compared to a pure culture of *C. carboxidivorans* (Bäumler et al., 2021). This is in agreement with the results obtained by Diender et al. (2019), in which the presence of *C. kluyveri* modifies the metabolism of acetogens towards solventogenesis for the benefit of alcohols production. This is due to the continuous elimination of ethanol by *C. kluyveri*, thus displacing the flux of electrons from CO towards ethanol in the acetogen. Other authors also associate this production of alcohols with a stress response to the presence of high amounts of acids in the non-dissociated form, which is more toxic, and whose proportion is higher at low pHs (Diender et al., 2016). However, one of the major bottlenecks of this technology is the pH mismatch between the two types of microorganisms, as acetogenic bacteria usually have an optimum growth pH close to 6, while that of *C. kluyveri* is 6.8, that is near neutrality. In any case, the research conducted by Ganigué et al. (2016) with a mixed culture shows that the lower pH threshold that allows solventogenesis and survival of *C. kluyveri* simultaneously is still as low as 4.7–4.8. On the other side, though optimal pH values of most acetogens are acidic, the optimal pH for solventogenesis in *C. aceticum* is close to pH 6.8 (Arslan et al., 2019), which is also the optimal pH of *C. kluyveri*. In that sense, a recent study showed that co-cultures of *C. aceticum* and *C. kluyveri* allow overcoming the pH mismatch issue between gas-fermenting acetogens and chain-elongating bacteria (Fernández-Blanco et al., 2022). In that same study, it was also observed that although some acetogens, such as *C. aceticum*, cannot produce higher alcohols (e.g., *n*-butanol) directly from C_1-gases, they can accumulate such higher alcohols instead from *n*-butyrate (Fernández-Blanco et al., 2022).

Nevertheless, although this strategy of indirect alcohol production by combining two bioconversion platforms is interesting to be optimized, it would be necessary to maintain carboxylate production while maintaining a low concentration of carboxylic acids to circumvent bacterial growth inhibition (Richter et al., 2013). It is also feasible to use a reactor configuration where chain elongation is achieved using pure cultures, that is using two different reactors (Figure 1c2). Gildemyn and co-workers used this configuration of reactors to produce medium-chain carboxylates from *C. kluyveri*, using filtered ethanol-rich medium from *C. carboxidivorans* P7 broth fed with real syngas effluent (Gildemyn et al., 2017).

METABOLIC ENGINEERING IN *CLOSTRIDIUM SPP.* TO ENHANCE BIOFUELS PRODUCTION

In recent years, many advances have been made in metabolic engineering to genetically modify different strains of the *Clostridium* genus to make them behave phenotypically different from the wild type, improving bioalcohol production. For example, Köpke et al. (2010) developed a genetic system for *C. ljungdahlii* by introducing the genes of the butanol synthesis pathway of *C. acetobutylicum*. In a batch syngas fermentation study, a maximum butanol concentration of 0.15 g/L (2 mM) was detected during the mid-growth phase which then decreased to very low concentrations. On the other hand, in silico metabolic engineering strategies were performed in *C. ljungdahlii* to stimulate the production of non-native products such as butanol and butyrate and to enhance the synthesis of ethanol, lactate and 2,3-butanediol from syngas (Chen & Henson, 2016). For this purpose, a genome-scale reconstruction of the metabolome of *C. ljungdahlii* (Nagarajan et al., 2013) was employed. According to the OptKnock computational framework, when ACKr (acetate kinase) and FRNDR2r (ferredoxin:NAPD reductase) catalysed reactions are eliminated, they resulted in a high butanol flux with only a 30% reduction in growth rate compared to the wild-type strain. Based on the same model, the ethanol flux could be almost doubled by suppressing the PTAr (phosphotransacetylase)-catalysed reaction that produces acetyl phosphate or the ACKr-catalysed reaction that produces acetate; however, this would compromise the growth rate by 35%. On the other hand, it was determined that by 4 deletions or elimination of the reactions catalysed by ALCD2x (alcohol dehydrogenase, ethanol), FRNDPR2r, ACKr and LDH_D (D-lactate dehydrogenase), a mixed product consisting of 2,3-butanediol and ethanol and an increase in 2,3-butanediol flux by 271% compared with 3 delections or elimination of the reactions catalysed by ALCD2x, FRNDPR2r and ME2 (malic enzyme, NADP) is achieved. In the case of *C. autoethanogenum*, inactivation of aldehyde/alcohol dehydrogenase (AdhE) has been shown to result in up to 180% higher autotrophic ethanol production and 38% less accumulation of the unwanted by-product acetate (Liew et al., 2017). Also, for the *C. carboxidivorans* strain grown in syngas, AOR, AdhE2 and FNR were overexpressed along with AdhE2 or AOR in a recent study (Cheng et al., 2019). The results showed that the strain overexpressing AdhE2 produced about 50% more ethanol than the wild-type strain. In the case of the strain overexpressing AdhE2 and FNR, it produced about 18% more butanol and 22% more ethanol.

FUTURE PERSPECTIVES

Syngas fermentation appears as an attractive mean for the production of valuable biofuels from low-cost gaseous emissions. In turn, the advantages over other thermochemical processes (i.e. Fischer-Tropsch synthesis) are several, such as its higher tolerance to impurities (e.g. sulfur compounds), lower operating temperatures and pressures which allows almost complete gas conversion, greater versatility in terms of workable mixtures of CO, CO_2 and H_2, and higher product yields. However, there are a number of shortcomings that should be overcome. For example, low cell densities and low gas–liquid mass transfer rates are the main limitations of this technology, thus generating low productivities (Perret et al., 2022). Likewise, inhibition of the solventogenic biocatalyst could be affected by many factors, for example non-optimal pH values, metabolites accumulation and presence of oxygen.

In order to optimize this bioconversion process, it is essential to thoroughly study the biology behind the syngas fermentation process in order to guide the design strategies (Latif et al., 2014). However, one of the biggest challenges in this field is the lack of tools to better understanding the syngas fermenting microorganisms. Therefore, one of the target objectives would be, through strain engineering, to obtain the desired production phenotypes (i.e. alcohols) by making use of such genetic tools and the latest advances in whole genome sequencing. Besides, it is possible to achieve phenotypes with better suitability for syngas fermentation through adaptive laboratory evolution (ALE), thus resulting, for example, in optimal growth of the microorganism during fermentation and tolerance to high concentrations of substrates and products (e.g., CO and ethanol, respectively) (Latif et al., 2014).

Further improvements to be addressed are the optimization of culture media, given the substantial impact that reducing agents and metal ions have on syngas utilization (Cheng et al., 2019) and the evolution of strains able to survive in minimal medium. In this way, it has been shown that the addition of exogenous agents such as activated carbon and nanoparticles improves the conversion efficiencies of CO and H_2 towards the product due to the increased gas solubility (Atiyeh et al., 2016).

Syngas composition, pressure and mass transfer are key points affecting fermentation titre, productivity and ethanol/acetate ratio. CO provides more energy than H_2 to the cells, so higher partial pressures of CO can lead to higher ethanol production, at the expense of the inhibitory effect it may cause at higher concentrations. Also, given the low water solubility of these gases, an increase in gas pressure and an improvement in mass transfer are relevant to improve solvent production. This is closely related to the design of the reactor used, since this has an important effect on the mass transfer coefficient, k_{La}. For instance, most syngas fermentation studies, at research level, use continuous stirred tank reactors (CSTR), although achieving sufficient mixing in fermenters with a volume of more than 500 m^3 involves very high energy inputs. Another alternative would be bubble columns, especially because of their simple and cost-effective design and low energy cost, together with the fact that it is possible to operate with large working volumes, even though the mixing of the liquid phase in this type of reactor is limited. On the other hand, biofilm reactors and membrane reactors allow higher cell concentrations to be achieved thanks to biocatalyst fixation, which prevents wash-out, and enables gas–liquid transport at high rates. Hence, re-designing the reactor configuration to achieve process scalability are issues that should be addressed while maximizing the value of k_{La} (Stoll et al., 2020). Optimization of the different operational parameters of the system is also necessary. For instance, the stirring speed helps to increase the rotational movements of the fermentation broth, so that the released gas bubble reaches a higher lateral speed, which permits it to move along a longer path prior to diffusing into the headspace of the reactor. Reactor stirring also aids in breaking the bubble, improving gas retention time and increasing the gas–liquid interfacial area for mass transfer (Roy et al., 2016).

Also, separating the products from the fermentation broth is an energy-intensive, and therefore costly, process. For this reason, it is important that future research is steered in this direction, to develop efficient extraction systems for the targeted products (i.e. alcohols). Satisfactory results have been seen during this last decade with membrane-based extraction methods to improve ethanol productivity (Roy et al., 2016), liquid–liquid extraction methods for hexanol removal (Oh et al., 2023), and in-line product extraction through gas stripping and product condensing for a mixture of alcohols and fatty acids (C_2-C_8) (Richter et al., 2016).

Finally, the isolation of a new chain elongator microorganism that has a growth pH close to 5 could be of great relevance (Richter et al., 2016). In this way, the range of possibilities to work with acetogens whose pH of solventogenesis is around that value would be widened, given the optimal neutral pH of the most common chain elongator, that is *C. kluyveri*, which is too high for most acetogens.

To sum up, syngas fermentation is a powerful tool for obtaining biofuels, but it needs further research, as it is a rather recent research field. On the one hand, as already indicated, it presents multiple advantages, such as the fact that it can work at low pressures and temperatures, which allows almost complete gas conversion. By the way, the aforementioned limitations of this approach should be tackled to make this technology even more effective for solvent production.

Regarding the future of biofuels, it is also worth mentioning that the National Ignition Facility (NIF), in an inertial confinement nuclear fusion experiment,

achieved a positive gain factor in December 2022, meaning that the nuclear fusion process produced more energy than was put into the fuel (Lawrence Livermore National Laboratory, 2022). Due to the novelty and also potential impact of this finding, it is difficult to draw conclusions on the long-term prospects for the use of biofuels. However, in the short and medium term, and thanks to several recent European directives such as Directive (EU) 2018/2001 (European Union, 2018a) and Directive (EU) 2018/2002 (European Union, 2018b), the European Union is committed to the use of biofuels as renewable energy sources, as part of the 2020–2030 agenda. Therefore, if well-funded and accompanied by legislation, the technology developed to produce biofuels from syngas and other substrates has clear present and future commercial potential, and the use of biofuels would increase in the coming years, similarly as it has been doing in recent years in the European Union.

CONCLUSIONS

Based on different pathways and bioprocesses, biofuels can efficiently be produced from C_1-gases by Clostridia and other related bacteria. Some of those processes are relatively mature and have even been scaled up to (pre)-commercial scale. However, they still deserve further optimization, and cost-efficiency compared with conventional oil-related fuels can still be improved. This is the case of ethanol production from C_1-gases through the WLP pathway. Ethanol separation requires intensive energy for its separation and purification, significantly increasing costs. The potential of HBE fermentation and co-culture processes, such as chain elongation, to produce longer chain alcohols, that is butanol or hexanol, is an attractive alternative technology. Since yields are still low in these processes, optimization is necessary to make them potentially effective at an industrial scale. Though limited results have been reported, metabolic engineering is another potential alternative to improve alcohol yields and productivities.

AUTHOR CONTRIBUTIONS

Carla Fernández-Blanco: Writing – original draft (equal); writing – review and editing (equal). **Raúl Robles-Iglesias:** Writing – original draft (supporting); writing – review and editing (supporting). **Cecilia Naveira-Pazos:** Writing – original draft (supporting); writing – review and editing (supporting). **María C. Veiga:** Funding acquisition (equal); resources (equal); supervision (supporting); writing – review and editing (equal). **Christian Kennes:** Funding acquisition (equal); project administration (lead); resources (equal); supervision (lead); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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