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Enhanced solventogenesis in syngas bioconversion: Role of process parameters and thermodynamics

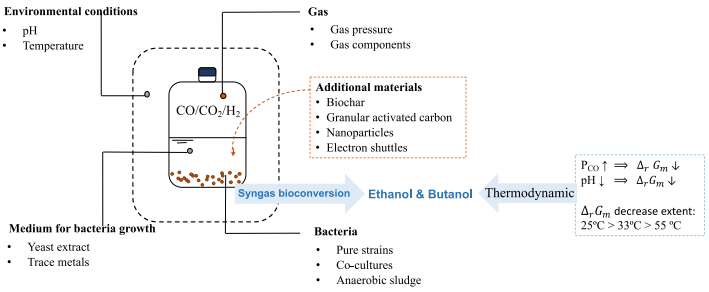
Yaxue He [a,b,*], Christian Kennes [b], Piet N.L. Lens [a]

[a] National University of Ireland Galway, H91 TK33, Galway, Ireland
[b] Chemical Engineering Laboratory, Faculty of Sciences and Center for Advanced Scientific Research - Centro de Investigaciones Científicas Avanzadas (CICA), BIOENGIN Group, University of La Coruña (UDC), E-15008, La Coruña, Spain

HIGHLIGHTS

- Process parameters regulating alcohols production from syngas are reviewed.
- The biocatalytic activity of pure and mixed cultures are compared.
- Thermodynamic calculations of CO gas pressure and pH effect on alcohol production.
- Potential strategies for enhanced longer chain alcohols production are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

Biofuels, such as ethanol and butanol, obtained from carbon monoxide-rich gas or syngas bioconversion (solventogenesis) are an attractive alternative to traditional fermentation processes with merits of no competition with food production and sustainability. However, there is a lack of comprehensive understanding of some key process parameters and mechanisms enhancing solventogenesis during the fermentation process. This review provides an overview of the current state of the art of the main influencing factors during the syngas fermentation process catalyzed by acetogenic species as well as undefined mixed cultures. The role of syngas pressure, syngas components, fermentation pH, temperature, trace metals, organic compounds and additional materials is overviewed. As a so far hardly considered approach, thermodynamic calculations of the Gibbs free energy of CO conversion to acetic acid, ethanol, butyric acid and butanol under different CO pressures and pH at 25, 33 and 55 °C are also addressed and reviewed. Strategies for enhancing mass transfer and longer carbon chain solvent production are considered as well.

1. Ethanol and butanol production from gas fermentation

Traditional fossil fuel utilization induces increased carbon emissions, further resulting in environmental problems such as climate change and global warming (Latif et al., 2014). On the other hand, the increasing demand of energy and the gradual depletion of fossil fuels renders the development of renewable energy necessary and emergent (Devarapalli and Atiyeh, 2015; Gavala et al., 2021). Compared to the traditional

* Corresponding author. Department of Microbiology and Ryan Institute, NUI Galway, University Road, H91 TK33, Galway, Ireland.
E-mail address: Y.HE2@nuigalway.ie (Y. He).

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heterotrophic ABE (acetone-butanol-ethanol) fermentation from sugars, syngas fermentation (as an alternative to carbohydrates) for biofuel production has become a promising technology for valuable chemicals production such as ethanol and butanol (Bajón Fernández et al., 2017; Sadhukhan et al., 2016; Fernández-Naveira et al., 2017a).

Syngas is a mixture of gases mainly containing CO, CO_2 and H_2 and is generated by thermal gasification of biomass, organic wastes from agriculture, cellulose, semi-cellulose or coal, among others (Fig. 1). The gasification of these different kinds of feedstocks takes place under high temperature and pressure in a gasifier, with a gasifying agent such as, air, oxygen or steam (Sansaniwal et al., 2017). The syngas composition is influenced by a variety of parameters, such as the gasifier type and its operating mode (Sansaniwal et al., 2017). Syngas fermentation is not only meaningful for biofuel production, but also for reduction of the carbon emissions, e.g. CO_2, that can be converted to CO by electrolysis, yielding a mixture of CO and H_2 and unreacted CO_2 that can be further used in the syngas fermentation process (Stoll et al., 2020). Ethanol and butanol are both valuable fuels, for instance, ethanol can be mixed at a 10% ratio with gasoline for energy while butanol has a similar energy content as gasoline and a higher commercial value than ethanol and can potentially replace and reduce fossil fuel consumption.

Bioconversion of syngas offers several advantages, such as high product selectivity, greater product titers and low energy costs (Klasson et al., 1992; Munasinghe and Khanal, 2010). A handful of acetogens have been isolated which are able to use syngas as their substrate for growth. Among them, several *Clostridium* spp. are the main functional species due to a special enzyme, carbon monoxide dehydrogenase (CODH), which allows them to overcome the toxicity of carbon monoxide. *Clostridium* spp. may convert carbon dioxide and carbon monoxide via the Wood-Ljungdahl pathway (WLP) (Fig. 2), leading to the production of acetic acid, butyric acid, ethanol and butanol or even occasionally hexanoic acid and hexanol (Bengelsdorf et al., 2013; Kennes-Veiga et al., 2022a). The WLP is the most effective, non-photosynthetic carbon fixation pathway (Jones et al., 2016). In the WLP, CO is first converted to CO_2, then 2 mol of CO_2 are reduced, using H_2 as the electron donor, to form 1 mol of acetyl-CoA (Ragsdale, 1997) (Fig. 2). Two essential enzymes are involved in the pathway, i.e., carbon monoxide dehydrogenase (CODH) and acetyl coenzyme A (acetyl-CoA) synthase (ACS). CODH catalyzes the reversible oxidation of CO to CO_2, and ACS combines with CODH to form a CODH/ACS complex for acetyl-CoA fixation (Liew et al., 2016).

Biofuel production from syngas has received increasing attention only since the last decade and this process is far less understood than anaerobic sugar fermentation. Recent reviews on biofuel production studied syngas fermentation, however, thermodynamic aspects of CO conversion were seldom reported (Sun et al., 2019). Mixed cultures of *Clostridium* spp. have been investigated and reviewed but undefined mixed cultures such as anaerobic sludge were generally excluded (Cui et al., 2020) and the product considered was generally limited to one alcohol such as butanol (Pinto et al., 2021). Considering its huge potential for sustainable biofuels production and its promising applications, this review discusses the mechanisms of biofuels production, identified process parameters for solventogenesis and explores future strategies for enhanced alcohol production. As shown in Fig. 3, four main aspects have been integrated, i.e., i) gas phase, including gas pressure and components, ii) medium composition including yeast extract and trace metals, iii) environmental conditions including pH and temperature and iv) microorganisms including pure cultures, co-cultures and anaerobic sludge (Fig. 3). This review also looks at more recent approaches to use i) additional materials such as biochar, granular activated carbon, nanoparticles and electron shuttles and ii) mixotrophy to enhance solventogenesis.

2. Solventogenesis in gas fermentation

Syngas fermentation for biofuels production is considered to comprise two stages: acetic acid accumulation (acetogenesis) and alcohol production (solventogenesis) (Fernández-Naveira et al., 2017a). Acetogenesis releases ATP for cell growth and metabolism; however, solventogenesis generates less net energy than acetogenesis and is thus often deemed as a passive choice by acetogens (Richter et al., 2016a).

The mechanism of solventogenesis remains not fully understood although solventogenesis has been shown to be triggered by stressful conditions such as low pH or nutrient limitation (Fernández-Naveira et al., 2017a). There are several hypothesis and explanations related to the mechanism in the literature. One is dealing with pH decrease (Ganigué et al., 2016): when the pH drops along with the accumulation of acids, undissociated acids are able to cross the cytoplasmic membrane by diffusion. To avoid cell damage or death due to the protons released by dissociated acetic and butyric acids, the microorganisms will convert the acids into neutral charged solvents (Fernández-Naveira et al., 2017a). Therefore, solventogenesis is a strategy for preventing a further pH drop at the expense of a lower ATP yield and risk of toxicity from the solvents. Another explanation is from an energetic viewpoint (Liu et al., 2020a): ethanol production is the preferred NADH sink and can relief energy and sustain cell survival via ethanol oxidation during the stationary phase.

In the acetogenesis stage, the high undissociated acid accumulation

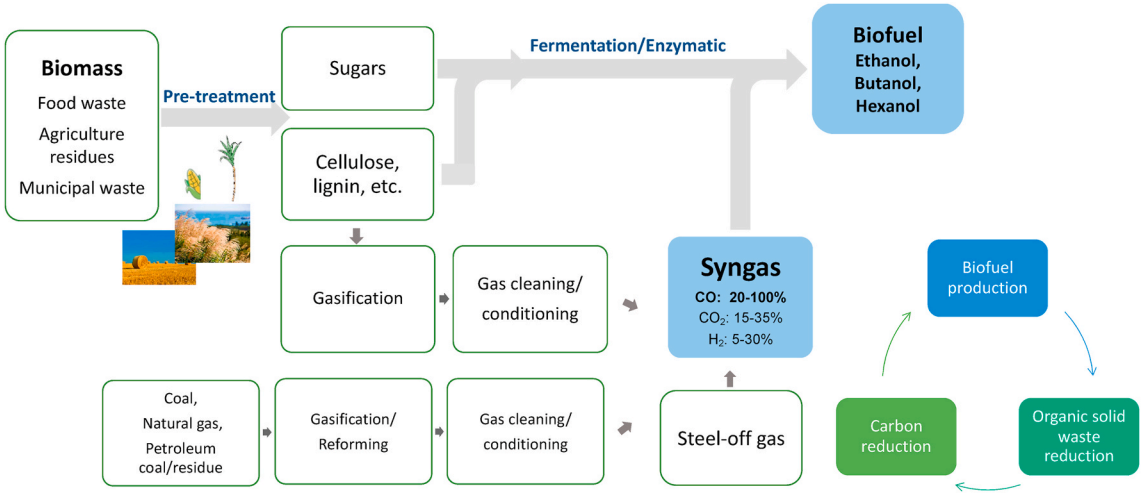

Fig. 1. Biofuel production from a variety of resources using direct fermentation of the organic feedstock, eventually after a pretreatment or indirect fermentation of the feedstock to syngas (CO, H_2 and CO_2).

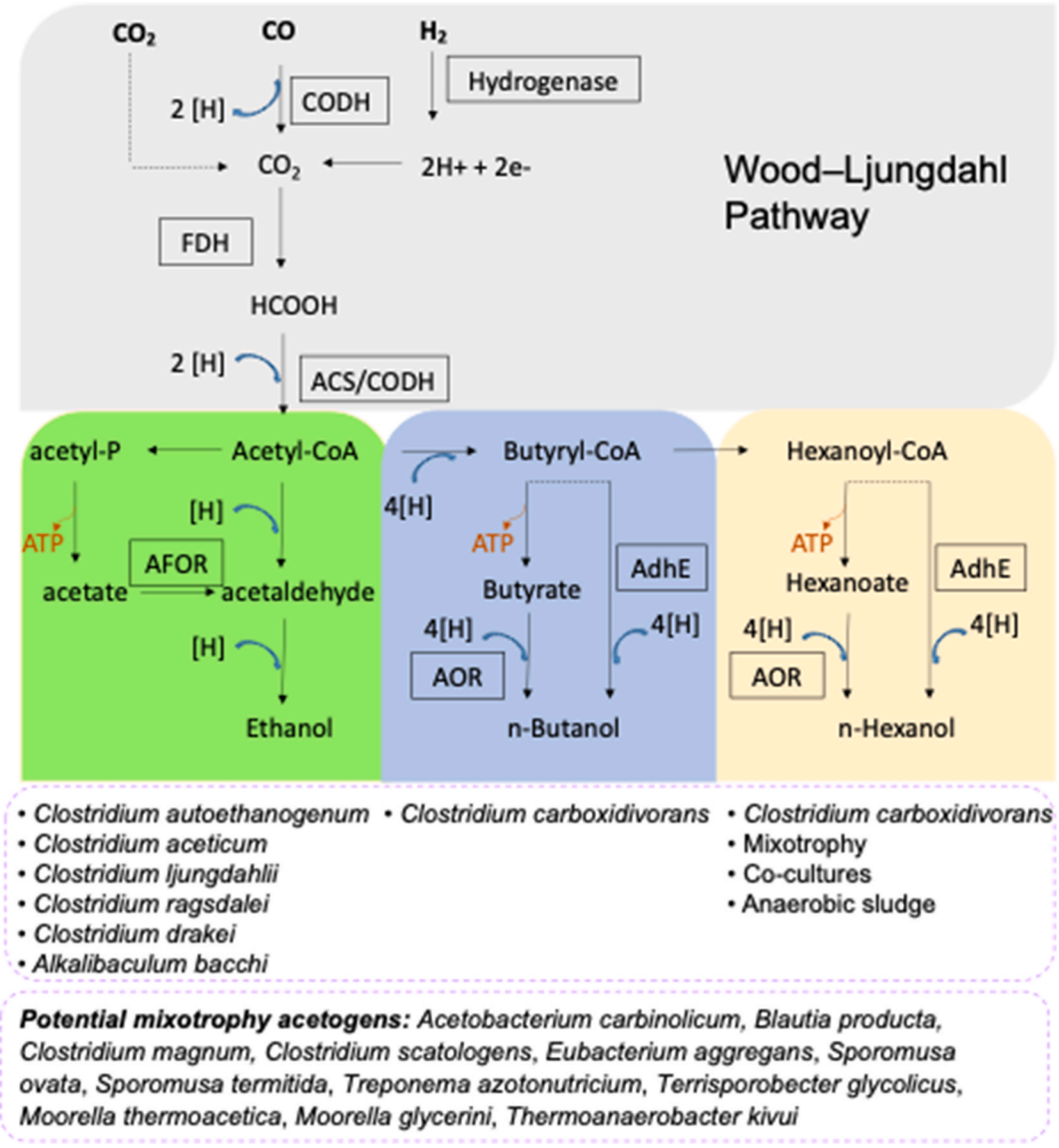

Fig. 2. Wood–Ljungdahl pathway and metabolites formation from acetyl-CoA, with the corresponding metalloenzymes. The corresponding microorganisms under Acetyl-CoA, Butyryl-CoA and Hexanoyl-CoA represent their ethanol, butanol and hexanol production ability. The pathway was adapted from previous publications (Norman et al., 2018; Kennes et al., 2016).

rate or concentration (37–54 mM or above) under uncontrolled pH conditions might cause an 'acid crash' (Mohammadi and Mohamed, 2014), which makes the cultures lose their alcohol conversion ability and further induces failed alcohol production (Wang et al., 2011; Mohammadi and Mohamed, 2014). Therefore, under uncontrolled pH conditions, slowdown of the acid accumulation rate could improve solventogenesis, which could be achieved by slowing down the metabolism/growth of bacteria, such as in fermentation under low temperatures and nutrient limitations.

Alcohols are considered as by-products produced by resting non-growth stage cells (Cotter et al., 2009). However, recent research revealed ethanol production occurs at the exponential growth stage as well. In fact, solventogenesis at non-growth or exponential stage varies with different fermentation conditions, such as the partial pressure of CO. Growth-associated ethanol production was reported when the CO partial pressure was increased to 2 atm in *C. carboxidivorans* $P7^T$(Hurst and Lewis, 2010). Recent research on *Clostridium ljungdahlii* revealed that ethanol synthesis is a preferred strategy to maintain the redox balance via $NADH/NAD^+$ recycling during autotrophic growth and ethanol was formed during the exponential phase, closely accompanied by biomass production under CO/CO_2 (vol/vol, 80/20) supply (Liu et al., 2020a).

3. Microorganisms

Three types of inocula have been studied and described in the literature (Table 1):

i) Pure cultures, mainly *Clostridium* spp.
ii) Defined co-cultures, which may consist in a combination of autotrophic and heterotrophic acetogens (Fig. 4a). Indeed, *C. autoethanogenum* can convert CO or syngas to ethanol and acetate, but when co-cultured with *Clostridium kluyveri*, the co-culture may end up producing butanol or hexanol with CO as reducing power, not found in any of those individual strains (Diender et al., 2016). This same was also observed in a co-culture of *Clostridium aceticum* and *C. kluyveri* (Fernández-Blanco et al., 2022).
iii) Undefined mixed cultures such as anaerobic sludge, sediments or animal manure (Chakraborty et al., 2019). The latter have also

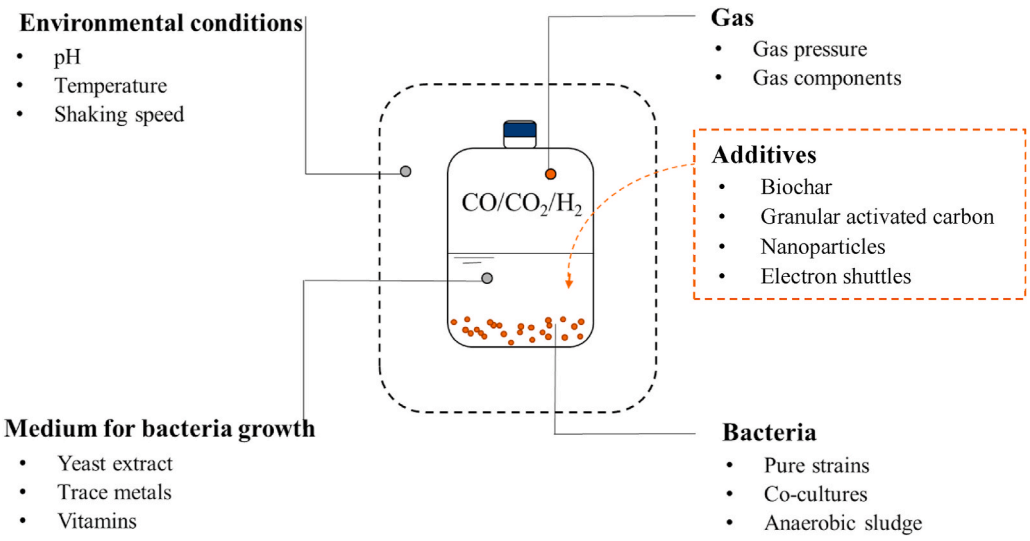

Fig. 3. Process parameters influencing solventogenic syngas fermentation.

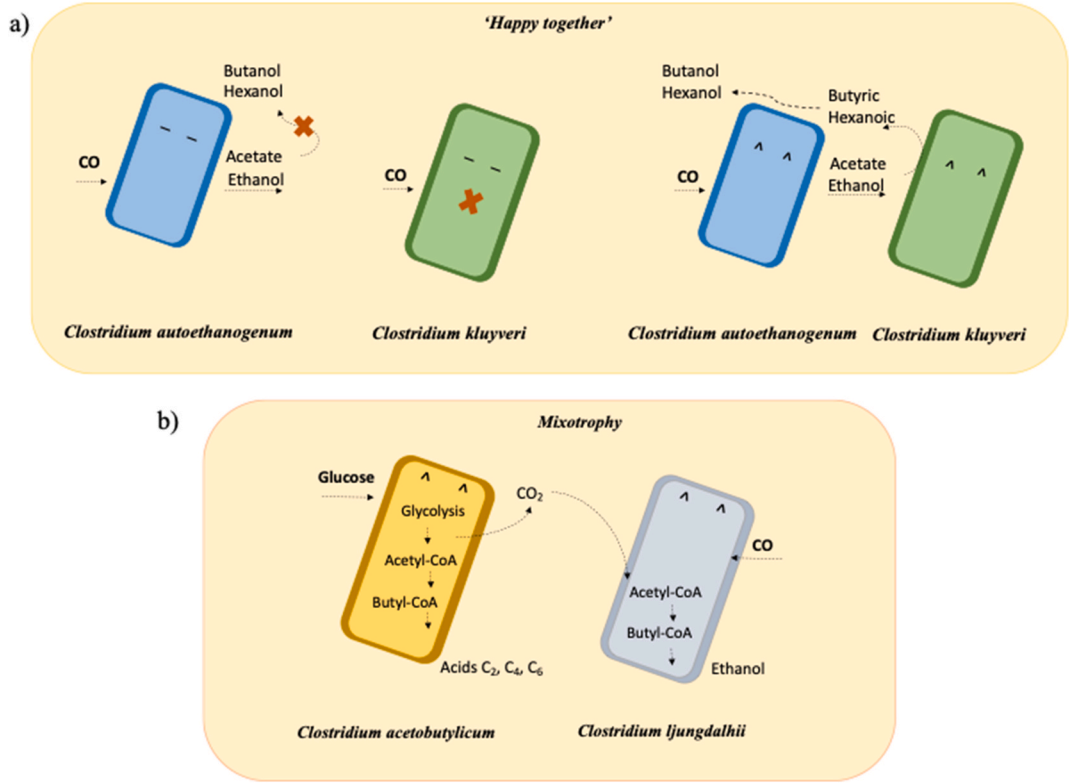

Fig. 4. Diagram of the **a)** co-culture of *C. autoethanogenum* and *C. kluyveri* using CO as the substrate and **b)** *C. acetobutylicum* and *C. ljungdalhii* co-culture. *C. acetobutylicum* consumes glucose to produce solvents, while releasing CO_2 and H_2. *C. ljungdalhii* consumes H_2 and CO_2 for survival and growth producing acetate and ethanol through WLP.

been the source of enrichment and isolation of novel autotrophic species, e.g. *Acetobacterium wieringae* Strain JM grown at 1.70 bar CO (Arantes et al., 2020), *Clostridium* sp. AWRP grown on syngas (Lee et al., 2019), or species not known, until recently, to produce alcohols, such as *C. aceticum* (Arslan et al., 2019). *Clostridium butyricum,* an organic carbon utilizing *Clostridium* sp. has also recently been shown to produce ethanol using syngas ($CO/CO_2/H_2/CH_4/N_2$, 22.92/7.90/13.05/1.13/45.58) (Monir et al., 2020).

It is worth mentioning that limited studies have reported mixed culture C_1-gas fermentation for the production of solvents, contrary to assays with pure culture species, as shown in Table 1.

Pure cultures of solventogens are characterized by slow growth rates, low cell density and metabolites produced in large quantities generally contain no more than two carbons (Liu et al., 2014). For instance, *C. carboxidivorans* is the only reported butanol producer from syngas bioconversion in pure culture (Fig. 2). Instead, co-cultures provide a promising solution for enhancing ethanol and longer carbon chain solvents production (Fig. 4b). For example, in co-cultures of the

Table 1
Alcohol and organic acid concentrations, yields and productivities during syngas fermentation using various biocatalysts.

	Microorganism	Reactor	Gas	Volume (L)	T (°C)	Agitation (rpm)	Gas flow (mL/min)	Time (d)	pH	Highest alcohols (g/L)		Reference
										Ethanol	Butanol	
Pure strains	*Alkalibaculum bacchi* CP15	CSTR	$CO/CO_2/H_2/N_2$ (20/15/5/60)	3.3/7	37	150	NA	51	8.0	6.0	1.1	Liu et al. (2014)
	C. carboxidivorans P7	CSTR	100% CO	1.2/2	33	250	10	21	5.75, 4.75	5.55	2.66	Fernández-Naveira et al. (2016a)
		Batch	$CO/CO_2/H_2/Ar$ (56/20/9/15)	0.03/ 0.125	37–25	100	NA	5	NA	3.64	1.35	Shen et al. (2020)
		CSTR	CO/CO_2 (60/40)	1st 1/2 2nd 1.5/ 2.4	37	NA	5 L h^{-1}	6	6.0, 5.0	6.1	0.7	Doll et al. (2018)
	C. autoethanogenum	CSTR	100% CO	1.2/2	30	250	10	7	6.0, 4.75	0.9	NA	Abubackar et al. (2015)
	C. ljungdahlii	Batch	$CO/CO_2/H_2$ (20/ 20/5)	200/250	37	200	NA	24 h	6.7 with nanoparticles	0.3	NA	Kim et al. (2014)
		Batch	$CO/CO_2/H_2$ (20/ 20/5)	200/250	37	200	NA	60 h	10.9 with $CoFe_2O_4$	0.5	NA	Kim and Lee (2016)
	C. aceticum	CSTR	$CO/CO_2/H_2/N_2$ (30/5/15/50)	1.2/2	30	150	10	52	6.98	5.6	NA	Arslan et al. (2019)
	C. ragsdalei	Tricking bed reactor	$CO/CO_2/H_2/N_2$ (38/28.5/28.5/ 5)	1	37	NA	5	70	5.8–4.6	5.7	NA	Devarapalli et al. (2016)
Co-culture	*C. autoethanogenum & C. kluyveri*	Fed batch	CO	0.07/ 0.25	37	NA	NA	NA	NA	NA	NA	Diender et al. (2016)
Mixed cultures	Anaerobic sludge from industry wastewater treatment	CSTR	100% CO	1.2/2	33	120	10	42	6.2, 4.9	11.1	1.8	Chakraborty et al. (2019)
	CO adapted enriched sludge	Fed batch	100% CO	0.1/0.5	33	150	NA	29	5.0–6.3	11.8	1.0	He et al. (2021c)
	C. ljungdahlii and *C. kluyveri*	CSTR	$CO/CO_2/H_2/N_2$ (60/35/5)	1/2	37	NA	30–80	93	5.7–6.4	144.7 mmol $C{\cdot}L^{-1}$ d^{-1}	39.2 mmol $C{\cdot}L^{-1}$ d^{-1}	Richter et al. (2016b)

*NA-not applied.

CO-utilizing *C. autoethanogenum* and the non-CO utilizing *C. kluyveri, C. autoethanogenum* facilitates the growth of *C. kluyveri* using CO as carbon source and both butanol and hexanol were obtained in that co-culture (Diender et al., 2016).

Anaerobic sludges from wastewater treatment plants are suitable sources of microbial species such as methanogens, hydrogenogens and acetogens, capable of syngas to solvents conversion (Arantes et al., 2020). From a practical point of view, mixed culture fermentations are easy to implement at large scale due to their typical non-sterile bioreactor operating conditions (Charubin and Papoutsakis, 2019). On the other hand, the presence of a broad range of acetogenic organisms in mixed cultures can better withstand unfavorable environmental conditions, including their tolerance to a wide pH range for alcohol production (Liu et al., 2014).

4. Process parameters for enhanced alcohol production

4.1. Gas phase

4.1.1. Gas pressure

Contrary to traditional fermentation with soluble sugar substrates in liquid medium, syngas fermentation processes can be considered to occur in two steps in terms of substrate assimilation: first there is mass transfer of the gaseous substrates to the liquid phase followed by the utilization of the dissolved gas by the microbial cells. Thus, the fermentation process is partly limited by the mass transfer rate from the gas phase to the liquid phase, which is further related to the thermodynamic properties of the gaseous substrate. H_2 and CO both act as electron donors but based on the Gibbs free energy, CO delivers electrons at a much lower half cell potential and releases about 80 kJ/mol more Gibbs free energy to produce ethanol than H_2 (Table 2, Eq. (4) and (5)). However, H_2 will provide the reducing equivalents for only one third of the carbon in CO (Table 2, Eq. (2)). Besides, CO has a much greater Henry's law constant (K_H = 7.4 mM/atm) than H_2 (K_H = 0.78 mM/atm) and is thus more difficult to dissolve into the liquid phase, so the presence of both CO_2 and H_2 affect the fermentation products (Jack et al., 2019).

Concerning the effect of pressure, in a recent study, pressurization to increase the CO partial pressure (P_{CO}) from 0.35 to 2 atm increased cell growth by 440% and ethanol production was not observed when the P_{CO} was above 0.35 atm and the ethanol production was growth-associated while the P_{CO} increased to 2 atm in *C. carboxidivorans* $P7^T$ (Hurst and Lewis, 2010). The maximum ethanol/butanol production in *Clostridium carboxidivorans* was obtained with a liquid and gas volume ratio (V_L/V_G) of 0.28 at initial CO gas pressure of 1.7 atm, at constant CO partial pressure (P_{CO}) ranging from 0.5 to 2.5 atm, while the optimal P_{CO} for growth was 1.1 atm at a V_L/V_G of 0.28 and 0.92 in 120 mL serum bottles (Lanzillo et al., 2020). Besides, an increase in the initial pressure enhanced ethanol production in *C. autoethanogenum* when the CO pressure varied between 0.8 and 1.6 bar (Abubackar et al., 2012). Also, cultivation with the CO pressure maintained above 50 kPa in a co-culture of *C. autoethanogenum* and *C. kluyveri* resulted in less oxidation of ethanol back to acetate (Diender et al., 2016).

4.1.2. Gas composition

The gas composition, mainly in terms of the different CO, CO_2 and H_2 ratios, is another factor influencing solventogenic processes. For instance, a H_2/CO ratio of 0.36 led to a maximum ethanol to acetate ratio of 16, while increasing the H_2/CO value to 1.2 induced a lower ethanol to acetate ratio of 2.5 in *C. ljungdahlii* incubations (Gaddy, 2000; Jack et al., 2019). Besides, acetate production increased from 22.6 mM at a H_2/CO ratio of 0.5–35.2 mM at a H_2/CO ratio of 2, while ethanol production decreased correspondingly from 5.4 to 7.4 mM in *C. ljungdahlii* incubations. Therefore, the production of more reduced metabolites (such as ethanol) was favored by the increased CO concentration, while acetate was favored by increased H_2 concentrations during H_2/CO fermentation. CO conversion to acetic acid and ethanol yielded a higher Gibbs free energy and is more electron dense than H_2/CO_2 conversion since CO_2 has to be reduced to CO, which consumes additional electrons (from H_2), as observed in *C. ljungdahlii* (Jack et al., 2019). However, the presence of H_2 can, theoretically, enhance CO_2 reduction (Table 2) and enhance carbon utilization. If not enough H_2 is available, it can be endogenously produced via additional compounds such as zero valent iron (Fe^0). However, studies on syngas or CO_2 bioconversion by acetogenic bacteria with Fe^0 are very scarce and have hardly been reported (Bayar et al., 2022).

When grown on H_2/CO_2, *C. autoethanogenum* cell extracts had a higher enzyme concentration than in bacteria grown on pure CO (Mock et al., 2015). This was attributed to the fact that during growth on H_2/CO_2 the role of the enzyme is to catalyze the endergonic reduction of CO_2 to CO with reduced ferredoxin, whereas during growth on CO the function of the enzyme is to catalyze the exergonic oxidation of CO to CO_2 with ferredoxin (Mock et al., 2015).

CO_2 may also influence the process of ethanol and other alcohols conversion (Table 2, Eq. (10)). However, only few studies have reported on ethanol and alcohol metabolism during syngas bioconversion. Tan et al. (2014) investigated the butanol dehydrogenases during butanol degradation in the presence of 0.25% butanol in *C. ljungdahlii*. They found 0.05% butyrate at the end of growth under the effect of two butanol dehydrogenase encoding genes (CLJU_c24880 and CLJU_c39950). Supplementing media with carbon dioxide can be essential or greatly stimulatory to acetogenesis. The presence of carbon dioxide (CO/CO_2, v/v, 70/30) appeared to enhance both the acetic acid concentration and production compared to incubations with pure CO. CO_2 produced during solventogenesis from CO conversion can be involved in ethanol and butanol (re)oxidation to acetic acid and butyric acid, respectively, by enriched CO fermenting sludge (He et al., 2022a).

Table 2
Theoretical reactions of syngas fermentation into ethanol and butanol. ($\triangle G_r^0$ = Gibbs free energy of each reaction when the elements are in the most stable state under standard conditions (1 bar, 298.15 K, 1 moL·L^{-1}, pH 7)).

Products	Reactions	Gibbs free energy	
Acetic acid	$4CO(g) + 2H_2O(l) \rightarrow CH_3COOH(l) + 2CO_2(g)$	$\triangle G_r^0 = -154.6$ kJ/mol	(1)
	$2CO(g) + 2H_2(g) \rightarrow CH_3COOH$	$\triangle G_r^0 = -114.5$ kJ/mol	(2)
	$2CO_2(g) + 4H_2(g) \rightarrow CH_3COOH(l) + 2H_2O(l)$	$\triangle G_r^0 = -75.4$ kJ/mol	(3)
Ethanol	$6CO(g) + 3H_2O(l) \rightarrow CH_3CH_2OH(l) + 4CO_2(g)$	$\triangle G_r^0 = -217.4$ kJ/mol	(4)
	$2CO(g) + 4H_2(g) \rightarrow CH_3CH_2OH(l) + H_2O(l)$	$\triangle G_r^0 = -137.1$ kJ/mol	(5)
	$2CO_2(g) + 6H_2(g) \rightarrow CH_3CH_2OH(l) + 3H_2O(l)$	$\triangle G_r^0 = -96.5$ kJ/mol	(6)
	$CH_3COOH(l) + 2H_2(g) \rightarrow CH_3CH_2OH(l) + H_2O(l)$	$\triangle G_r^0 = -21.6$ kJ/mol	(7)
Butyric acid	$10CO(g) + 4H_2O(l) \rightarrow CH_3CH_2CH_2COOH(l) + 6CO_2(g)$	$\triangle G_r^0 = -420.8$ kJ/mol	(8)
Butanol	$12CO(g) + 5H_2O(l) \rightarrow CH_3CH_2CH_2CH_2OH(l) + 8CO_2(g)$	$\triangle G_r^0 = -486.4$ kJ/mol	(9)
Ethanol oxidation	$2CH_3CH_2OH(l) + 2CO_2(g) \rightarrow 3CH_3COOH(l)$	$\triangle G_r^0 = -32.2$ kJ/mol	(10)
Butanol oxidation	$2\ CH_3CH_2CH_2CH_2OH(l) + 2\ CO_2(g) \rightarrow 2\ CH_3CH_2CH_2COOH(l) + \ CH_3COOH(l)$		(11)

4.2. Fermentation conditions

4.2.1. pH

pH can significantly affect both the growth rate and product formation of microorganisms. During acetogenesis, due to the acid accumulation, the external pH starts to drop. To prevent a further drop in pH, an organism may begin to produce neutral charged solvents (Padan et al., 1981; Cotter et al., 2009). It is worth noting that the intercellular pH is higher than the external pH due to a proton translocation process catalyzed by a membrane-bound ATPase (Gottwald and Gottschalk, 1985). Some researchers (Abubackar et al., 2012) investigated the effect of the initial pH (4.75–5.75) on biological solvent production by *C. autoethanogenum* DSM 10061 and also used the Minitab analysis at a two level four factor (2^4) and found that lowering the pH resulted in the production of more reduced compounds such as ethanol. Therefore, lowering the pH facilitates the production of highly reduced products such as ethanol. However, lowering the pH also causes a decrease in electron and carbon flow from the substrate towards the cell mass and reduces the overall productivity of the process (Worden et al., 1989; Phillips et al., 1993).

The optimal pH for solventogenesis varies depending on the strain and is around 4.75 for *Clostridium autoethanogenum* (Abubackar et al., 2015, 2016; Fernández-Naveira et al., 2017b) and *C. ljungdahlii* (Infantes et al., 2020) and 5.0–5.8 for *C. carboxidivorans* P^7 (Fernández-Naveira et al., 2016a). In most pure culture studies, the maximum butanol concentration so far did generally not exceed 2 g/L. Instead, in a CO gas-fed bioreactor with an initial CO gas pressure of 1.8 bar, with regular pH adjustment to 5.7, accumulation of as high as 6.8 g/L butanol was reported, with a mixed enriched anaerobic sludge, which is also the highest butanol concentration reported in the literature and suggests the high potential of mixed cultures (He et al., 2022a).

4.2.2. Temperature

The growth temperature of most solventogenic *Clostridium* spp. ranges from 20 to 42 °C, with an optimum temperature of 37 °C (Naik et al., 2010). This is also one of the most common temperatures applied in the literature (Munasinghe and Khanal, 2010). A limited number of studies reported on less optimal temperatures, i.e. sub-mesophilic and psychrophilic conditions for solvents production from syngas (Kundiyana et al., 2011).

A higher ethanol and butanol production was obtained at 25 °C for syngas ($CO/H_2/CO_2/N_2$, 32/32/8/28, v/v) fermentation by *C. carboxidivorans* compared to 37 °C by avoiding the 'acid crash', while the pH dropped below 4.8 at 25 °C (Ramió-Pujol et al., 2015). An 'acid crash' and lower ethanol production was also observed at 37 °C compared to 25 °C in H_2/CO_2 fermentation by anaerobic granular sludge (He et al., 2021a). Although higher temperatures have a negative impact on the solubility of CO and H_2, which also decreases the mass transfer rate of these gases to the cells, this negative part could be neglected compared to the positive role of the non-acid phenomenon at lower temperatures (Henstra et al., 2007). He et al. (2021b) compared sub-mesophilic (18, 25 and 30 °C) conditions for ethanol production from H_2/CO_2 and concluded 25 °C gave higher ethanol yields than at 18 and 30 °C. To achieve both high cell growth and alcohol production, two-step temperature (TST) cultures were developed. For instance, 37–25 °C and 37–29 °C fermentation could overcome cellular agglomeration at 37 °C and low biomass growth at 25 °C and achieve a higher alcohol production and yield. Gene expression revealed the carbon-fixation pathway at 37 °C and product biosynthetic pathway at 25 °C (Shen et al., 2020).

The exploration of solventogenesis under thermophilic conditions via syngas fermentation is seldom reported. This is partly due to the mesophilic character of most *Clostridium* spp. since their growth temperature is up to 42 °C. Shen et al. (2018) reported acetate, butyrate and caproate production by *Clostridium* under mesophilic conditions, while acetate was the main product with *Thermoanaerobacterium* as the main microorganism under thermophilic conditions from syngas (CO/H_2, v/v, 40/60) fermentation in hollow-fiber membrane biofilm reactors inoculated with anaerobic sludge.

Acetate productivity was also enhanced under thermophilic (55 °C) conditions with anaerobic sludge using syngas (H_2/CO_2, v/v, 60/40) as the substrate with 10 mmol L^{-1} bromoethane sulfonate (BES) to inhibit methanogenesis (Wang et al., 2017).

Alves et al. (2013) studied the enrichment of anaerobic syngas-converting bacteria for more than one year using municipal solid waste seeded sludge as the inoculum under thermophilic conditions (55 °C) and reported that *Desulfotomaculum*, *Thermincola*, *Caloribacterium* and *Thermoanaerobacter* are associated with syngas and/or CO conversion in thermophilic enrichment cultures. Similarly, formate accumulation was obtained from H_2/CO_2 (80/20, v/v) and 300 mM $KHCO_3$ at 66 °C by *Thermoanaerobacter kivui* LKT-1 (Schwarz and Müller, 2020).

4.3. Medium composition

4.3.1. Trace metals

Trace metals affect solventogenesis by influencing enzyme synthesis and activity. In the WLP (Fig. 1), formate dehydrogenase (FDH) is one of the key enzymes, transferring CO_2 to formate. It could be stimulated by addition of trace metals such as tungsten (W), selenium (Se) and molybdenum (Mo) (Yamamoto et al., 1983). The other key metalloenzyme related to Mo and W is alcohol dehydrogenase (ADH), catalyzing the reduction of acetyl CoA to ethanol (Chen, 1995; Andreesen and Makdessi, 2008).

At the same time these trace metals might exhibit different effects in different CO-metabolizing strains. For example, the ethanol/acetic acid ratio was 5–7 times higher when adding 0.075 and 0.75 μM W compared to a control medium in CO bioconversion by *C. autoethanogenum* (Abubackar et al., 2015). Studies with W and Se showed that the addition of 0.75 μM W lead to the highest ethanol to acetic acid ratio and was 173% higher than the ratio obtained with 1.44 μM Se.

Comparing to the 35 mM ethanol production with standard metal concentrations, the concentrations of ethanol increased to 180, 200, 60, and 90 mM with more optimized trace metal concentrations of 8.5, 35, 5 and 7 μM of Ni^{2+}, Zn^{2+}, SeO_4^{2-} and WO_4^{2-}, respectively, while boosting also *Clostridium ragsdalei* cell growth (Saxena and Tanner, 2011).

The presence of both W (0.68 μM) and Se (1.15 μM) allowed to accumulate high amounts of alcohols (8.0 g/L total alcohols and 3.0 g/L total acids) at pH 5.0 from syngas ($CO/CO_2/H_2/N_2$, 30/10/20/40, v/v) with *C. carboxidivorans* P7. Instead, the absence of W and Se induced organic acids (9.6 g/L) accumulation and almost no alcohol production (0.7 g/L). When omitting W, but not Se, as high as 11.3 g/L acids accumulated with poor alcohol production. Therefore, tungsten has been shown to play the major role for solventogenesis, while selenium seems to be less significant (Fernández-Naveira et al., 2019).

Ni and Fe are also two vital metals for the growth of *C. carboxidivorans* P7 during syngas ($CO/CO_2/H_2$, v/v/v, 50/35/15) fermentation (Han et al., 2020). Decreasing the molybdenum concentration from 55 to 23 μg/L dramatically promotes cell growth and alcohol synthesis, particularly for ethanol and butanol production (Han et al., 2020). The high molybdate concentration of 55 μg/L inhibited the butanol synthesis pathways and the acid re-assimilation pathway (Han et al., 2020). The expression levels of the acetate synthesis genes *ack* (Ccar_00695) and *pta* (Ccar_00690) were significantly downregulated in the 23 μg/L Mo incubation. The regulation of trace metals not only stimulated the production of alcohol, but was also a trigger of acid reuse or re-assimilation leading to the transition from acidogenesis to solventogenesis in *C. carboxidivorans* strain P7 (Li et al., 2018).

Besides the essential investigation of W and Mo, zinc was then also found to enhance ethanol production from syngas ($CO/CO_2/H_2$, v/v/v, 50/35/15) by *C. carboxidivorans* P7 via upregulation of the expression of *fdhII* and *bdh*, with the following production improvements: ethanol

3.02-fold, butanol 7.60-fold (0.4 g/L) and hexanol 44-fold (0.1 g/L) with 280 μM Zn^{2+} compared with the values in the control medium (7 μM Zn^{2+}). Although a considerable enhancement of the alcohol production was reached, the obtained concentrations of ethanol, butanol and hexanol were not significantly higher compared to other studies, reaching ethanol concentrations of around 2 g/L, butanol 0.4 g/L and hexanol 0.1 g/L, upon addition of 280 μM Zn^{2+} in the fermentation medium (Li et al., 2018).

Conversely, some trace metals may also have some negative effect on acetogenic bacteria and solventogenesis. In that sense, it was observed, in *C. ragsdalei*, that removing Cu^{2+} from the culture medium enhanced ethanol production by about 50% (Saxena and Tanner, 2011).

4.3.2. Yeast extract

To ensure a certain amount of acid production, an easy consumable sugar such as glucose and yeast extract as nitrogen source can be added to enhance cell growth during ethanol fermentation from C_1 gas (Chakraborty et al., 2019).

Yeast extract can be an important nitrogen source for microorganisms. Strong negative effects on the production rates, and a significant increase of the lag phase were found when the yeast extract concentration is below 0.5 g/L with a synthetic co-culture of *C. autoethanogenum* and *C. kluyveri* grown on carbon monoxide (Diender et al., 2016). In a bioreactor with continuous CO supply and with 1 g/L yeast extract at pH 5.75, the maximum biomass concentration obtained was 302.4 mg/L, which is comparable to the maximum cell mass concentration obtained at pH 6.0 (Abubackar et al., 2015a). 1 g/L YE addition, even without sugars such as glucose and fructose, improved cell growth significantly and shortened syngas fermentation by *C. carboxidivorans* P7 (Wan et al., 2017).

However, eliminating YE is found to enhance the ethanol production using *C. ljungdahlii* (Barik et al., 1988). Nevertheless, for this organism to provide structural integrity, a minimum concentration of 0.01% is necessary (Abubackar et al., 2011). Some authors (Abubackar et al., 2011) investigated the effect of the yeast extract concentration (0.6–1.6 g/L) on biological solvent production in *C. autoethanogenum* DSM 10061 and also using the Minitab analysis at a two level four factor (2^4) and found that lowering the YE concentration results in the production of more reduced compounds such as ethanol.

Other cheap nutrient sources such as cotton seed extract (CSE) and corn steep liquor (CSL) can also replace yeast extract to enhance ethanol production (Silveira et al., 2001). CSE with a cost of $ 0.91/kg is considerably cheaper than yeast extract ($ 183/kg). CSL, as a major by-product of the corn wet milling industry, comprises of a rich source of important nutrients (Hull et al., 1996). Kundiyana et al. (2010) examined the ethanol and acetic acid production using cotton seed extract (CSE) as the fermentation medium for *Clostridium* strain P11 and reported 2.66 g/L ethanol in the batch medium containing 0.5 g/L of CSE after 15 days fermentation. The standard fermentation medium of *Clostridium* strain P11 could be replaced with CSE, since CSE contains minerals and vitamins which are very similar to the standard fermentation medium.

Benevenuti et al. (2020) used a medium TPYGarg (12 g/L of tryptone, 12 g/L of peptone from gelatin, 7 g/L of yeast extract, 1.2 g/L of L-arginine, and 1 g/L of glucose), with 1.22 atm syngas ($CO/CO_2/H_2/CH_4/N_2$, 25/10.02/43.9/11.01/10.05) and *C. carboxidivorans*. A 5-fold higher ethanol and 2 fold higher butanol production was achieved at a 31% cost reduction compared to the standard medium ATCC 2713. Hongrae et al. (2021) found that 0.5 g/L yeast extract addition yielded the highest ethanol production compared to 0, 2.5 and 5 g/L yeast extract in *C. autoethanogenum*. Based on the results with 0.5 g/L yeast extract addition, 0, 0.5, 2.5 and 5 g/L tryptone or peptone were investigated for ethanol production as well. A maximum of 4.57 g/L and 4.23 g/L ethanol were produced in media supplemented with 0.5 g/L of tryptone and 0.5 g/L of peptone, respectively.

5. Strategies for enhanced ethanol, butanol or longer carbon chain alcohols production

Solventogenic syngas fermentation has received increased attention only since the last decade compared to the traditional sugar fermentation with about hundred years of research history (Kennes-Veiga et al., 2022a; 2022b). Hence, it is an emerging sustainable biotechnological process for biofuel production. Syngas bioconversion can be applied as an innovative biorefinery concept and can be based on the gasification of agriculture wastes, municipal solid waste or renewable materials, as well as to industrial emissions. The fundamental understanding of the fermentation process for enhanced solvent production still has many undiscovered aspects, such as i) how to maximize efficient bio-catalysis for enhanced ethanol and butanol production, in both pure and mixed cultures and ii) aspects such as reactor design, mass transfer, production of longer chain solvents and other valuable chemicals production as well as efficient solvent separation.

5.1. Defined versus undefined mixed cultures

Despite the limited number of identified and studied pure solventogenic strains, the application of syngas fermentation in pilot plants has been set up in a fermentation process developed by LanzaTech for ethanol production from steel mill gas with a patented *Clostridium* strain (Yu et al., 2015). However, mixed cultures have raised less attention yet for waste gas and syngas conversion, despite their broad use in wastewater based biorefinery approaches. He et al. (2021c) enriched *Clostridium* spp. that can produce high ethanol concentrations with low acetic acid accumulation over a wide pH range of 4.95–6.45, which illustrates the merits and adaptation to broad environmental conditions of mixed cultures compared to pure strains. The enrichment of syngas fermentation solventogenic acetogens, especially on CO, as demonstrated in He et al. (2021b), resulted in less contamination problems, e.g., by methanogens, compared to H_2/CO_2 due to the high CO toxicity.

5.2. Reactor design

5.2.1. Continuous stirred tank reactor (CSTR)

The continuous stirred tank reactor (CSTR) has been widely studied for syngas biotransformation to solvents at lab scale (Arslan et al., 2019; Mohammadi et al., 2012). The agitation favours large bubbles breaking into smaller ones, hence improving the gas-liquid mass transfer rate. The shift of high pH (e.g. pH 6) to low pH (e.g. pH 4.8) in the CSTR has been shown an efficient way to enhance ethanol production without acid accumulation in a pure culture study (Abubackar et al., 2015). However, this approach is not economically feasible for scaled-up production because high agitation rates consume high amounts of energy resulting in a costly approach (Shen et al., 2014a, 2014b).

5.2.2. Bubble column

A bubble column reactor without agitation but combined with microbubble diffusion could highly enhance the mass transfer rate (Datar et al., 2004). The gas is injected and distributed through the bottom by gas sparging thus good mixing can be achieved while avoiding high shear rates that might inhibit or damage microbial cells. A bubble column reactor can also be used as second stage for solvent fermentation with high cell concentrations reached in a first stage CSTR (Richter et al., 2013).

5.2.3. Biotrickling filter

A biotrickling filter or trickle bed reactor is a packed bed reactor in which liquid medium is flowing down while the gas can flow either downwards or upwards (Cassarini et al., 2019). The colonized packed bed acts as the solid catalyst for gas-liquid interactions (He et al., 2018). The power consumption of trickle bed reactors is lower than that of CSTR and some studies also found that trickle bed reactors showed a

higher efficiency than bubble columns and CSTR (Cowger et al., 1992). Although their use in syngas fermentation is so far limited, biotrickling filters have been used for decades, at full-scale, for air pollution control and treatment of industrial emissions (Kennes and Veiga, 2013).

5.2.4. Novel bioreactor designs

The fact that the low water solubility of CO limits the mass transfer rate between the gas and liquid phase results in low achievable cell density in fermentation media. To avoid this, membrane systems (Jin et al., 2008), attached growth bioreactors (Shen et al., 2014a, 2014b) and microbubble spargers in suspended-growth bioreactors (Muroyama et al., 2013) allow more efficient transfer rates. Research and developments made on novel bioreactor designs for air pollution control could also be used and applied to syngas fermentation (Kennes and Veiga, 2013), including the rotating packed bed biofilm reactor and monolithic biofilm reactor (Gunes, 2021). The hollow-fiber membrane biofilm reactor has been demonstrated for its efficient CO and H_2 utilization for longer chain fatty acids production by mesophilic anaerobic inocula (Sathish et al., 2019).

5.3. Mass transfer

Except for the process factors investigated in section 4, such as temperature, pH, trace metals and gas pressure and composition, in H_2/CO_2 conversion, some other equally important factors such as addition of materials that influence the mass transfer rate can further improve the solventogenic yield from CO or similar gases. Mass transfer issues are among the major differences between syngas fermentation and traditional sugar fermentation. Next to the difference in metabolic pathway, the gaseous substrate, especially the low solubility of CO and H_2 (Fernández-Naveira et al., 2017a), affect the alcohol production process design.

5.3.1. Adsorptive and conductive materials

To overcome the low solubility and enhance the mass transfer rate, additional materials such as biochar and nanoparticles have been studied recently (Sun et al., 2018a, 2018b; Yao et al., 2018).

Biochar produced from biomass pyrolysis gasification with high carbon percentage and porous structure, metals and functional groups has been widely used in soil improvement and as adsorbent (Sun et al., 2018a, 2018b). Syngas production during gasification, simultaneously produces biochar but the properties of biochar may vary in terms of alkalinity, pH buffering capacity, cation exchange capacity and electrical conductivity (Yao et al., 2018). Due to the different biochar properties, its effect on solventogenesis yielded different results. The nutrients and pH buffering function of biochar may reduce acid stress. Enhanced ethanol production was achieved in a medium with poultry litter biochar due to its rich mineral and metal content, while inhibited ethanol production was observed with switchgrass biochar addition and *C. ragsdalei* (Sun et al., 2018a). However, *C. carboxidivorans* entered the solventogenic phase in switchgrass biochar treatment earlier than other biochar treatments, resulting in the accumulation of more ethanol and butanol (Sun et al., 2018b).

With methyl-functionalized silica nanoparticles, ethanol production increased from 0.115 g/L to 0.306 g/L with *C. ljungdahlii* using CO, CO_2 and H_2 (v/v/v, 20/20/5) because of the enhanced mass transfer rate after addition of nanoparticles (Kim et al., 2014). The authors of the afore mentioned research also found that magnetic nanoparticles (SiO_2–CH_3) increased the dissolved concentrations of CO, CO_2 and H_2 by 224%, 78% and 143%, respectively and ethanol production increased from 0.156 g/L to 0.354 g/L in *C. ljungdahlii* using CO, CO_2 and H_2 (v/v/v, 20/20/5) as the substrate (Kim and Lee, 2016).

5.3.2. Electron shuttles

Exogenous electron shuttles such as neutral red, methylene blue, methyl viologen and benzyl viologen have been used to shift fermentative metabolism towards alcohol production (Yarlagadda et al., 2012). Even though the exact mechanism whereby electron shuttles regulate the fermentative pathway is not fully understood, adding them to bacterial cultures is an attractive strategy for shifting the direction of electron flow from generating organic acids to solvents and hydrogen. Yarlagadda et al. (2012) investigated the effect of electron shuttles on the end products using glucose as carbon source in *Clostridium* sp. BC1 and found that methyl viologen enhanced ethanol and butanol production by 28 and 12 fold, respectively, due to a shift in the direction of electron flow towards enhanced production of ethanol and butanol by reducing hydrogen production.

5.4. Longer chain solvents and other valuable chemicals

The enhanced butanol production by supply of endogenous butyric acid from glucose and CO, described in He et al. (2022b) implies that exogenous acids using CO as the gaseous substrate through mixotrophy and with mixed cultures can become a strategy for enhanced longer carbon chain alcohol production. Besides, ATP generation during syngas fermentation is rather limited compared to sugar fermentation, which further limits longer carbon chain alcohol production. Several trials have explored butanol and hexanol production by co-cultures in the literature. He et al. (2021c), observed an unknown small amount of hexanol from endogenous butyric acid and CO. However, more investigations on the conversion mechanisms should be done, such as how the interspecies electron transfer occurred.

5.4.1. Mixotrophy

Autotrophic growth faces the challenge of ATP limitation, but part of the ATP can be recovered in acetogenesis via substrate-level phosphorylation (Maru et al., 2018). ATP limitation induces slow cell growth and low cell densities and does not ensure enough energy generation for producing large quantities of metabolites with more than 2 carbons (Fast et al., 2015). One promising way to overcome the ATP limitation can involve another ATP generating process such as glycolysis from sugar fermentation, i.e. mixotrophy. Mixotrophy comprises organic and inorganic (CO, CO_2 and H_2) substrate fermentation by autotrophic acetogens. Meanwhile, the mixotrophy can also be reached by co-cultures of heterotrophic and autotrophic acetogens, such as *C. acetobutylicum* and *C. ljungdalhii* for longer carbon chain alcohol production (Diender et al., 2016). However, it is also worth to take into account that somewhat different metabolites could be produced, or at different concentrations, from heterotrophic compared to autotrophic fermentation, even with a same organism (Fernández-Naveira et al., 2017c; Arslan et al., 2021).

Mixotrophy enhanced carbon utilization since the released CO_2 during the heterotrophic fermentation can be involved in syngas fermentation by autotrophic acetogens. Simultaneously, cell growth could be enhanced due to excess ATP generation. More importantly, longer carbon chain alcohols (>4) production was achieved via reduction of endogenous long chain fatty acids from heterotrophic fermentation (He et al., 2022b).

A potential application of mixotrophy can be to combine carbohydrate-rich wastewater and syngas co-fermentation. However, this approach has not yet been reported for alcohol production, although it has been used for biohydrogen (Liu et al., 2020b) and volatile fatty acids (Liu et al., 2020c). On the other hand, from a toxicological viewpoint, longer chain alcohols showed higher toxicity to the cells and could cause low alcohol production; for example, the IC_{50} of butanol for *C. carboxidivorans* growth was 14.50 g/L after 48 h and IC_{50} for ethanol appeared to be very close to 35 g/L (Fernández-Naveira et al., 2016b). However, the commonly reported maximum ethanol and butanol concentrations are presently still quite lower than the toxicity values reported in the literature so far.

5.4.2. Exogenous electron acceptors and acids

Solventogenesis generally requires a minimum level of acetic acid production, and supplementation of acetate during acidogenesis was reported to result in a significant increase in acetone–butanol–ethanol production (Gao et al., 2016). Ethanol production was enhanced with exogenous ^{13}C-labeled acetate in *C. autoethanogenum* using 100% CO as gaseous substrate and simultaneously *aor* gene CAETHG_0102, *codh* gene CAETHG_3005, *adh* genes CAETHG_1841 and CAETHG_1813 were found to be highly up-regulated at higher acetate levels. Exogenous acetate can thus play an important role in solventogenesis (Xu et al., 2020).

Due to the two-stage pathway of syngas fermentation, some studies directly added acids such as acetic acid, expecting higher ethanol production (Perez et al., 2013). Besides, electron acceptors like nitrate have been shown to enhance carbon dioxide fixation by *C. ljungdahlii* (Emerson et al., 2019). 15 mM nitrate supplemented H_2 + CO_2 fermentation enhanced the yield of production of heterologous chemicals such as acetic acid and ethanol by boosting ATP production in *C. ljungdahlii* (Emerson et al., 2019). However, nitrate and nitrite can have a negative effect on both growth and alcohol formation in *C. carboxidivorans* under continuous gas supply (CO/CO_2, 80/20) (Rückel et al., 2021).

6. Thermodynamic calculations of Gibbs free energy of CO conversion to acetic acid, ethanol, butyric acid and butanol

6.1. Gibbs free energy calculations

The thermodynamics of syngas bioconversion processes are rarely studied or reviewed due to its complexity in processes involving biomass (Gildemyn et al., 2017). To simplify, the following Gibbs free energy calculations do not involve biomass. Gibbs free energy of formation is zero for the elements in their most stable state, e.g. CO (g), H_2 (g), CO_2 (g), acetic acid (l), under standard conditions (1 bar, 298.15 K, 1 moL·L^{-1}) (Franses, 2014). Gibbs free energies (Δ_rG_m) for the production of acetic acid, ethanol, butyric acid and butanol from CO were calculated from the respective standard Gibbs free energies ($\Delta_rG_m^\theta$) and the actual concentrations of reactants and products using Van't Hoff equation (Eq. (1)) (Thauer et al., 1977). If Δ_rG_m is negative then the system loses energy and does work. In this case, a lower Δ_rG_m means that the reactions are more favorable to proceed. Conversely, if Δ_rG_m is positive, then the system has to gain energy from the work that has been done by the surroundings (Schmitz, 2017). Thus, Δ_rG_m provides a valuable criterion for determining whether a reaction can occur spontaneously or not (Oubrahim and Chock, 2016).

It should be noted that for a thermodynamically unfavorable reaction (Δ_rG_m >0), if biological systems are involved, the overall Δ_rG_m of the pathway can become negative since such biological systems can formulate their metabolic pathways by coupling enzyme-catalyzed reactions (Oubrahim and Chock, 2016). The Gibbs free energy calculated in this review can be applied to compare the extent of the reaction system and if it is favorable with same substrate (e.g., CO) and microorganism.

For the reaction: $cC + dD = yY + zZ$

$$\Delta_rG_m = \Delta_rG_m^\theta + RTlnJ \tag{1}$$

where, $\Delta_rG_m^\theta$ is the standard reaction Gibbs energy, under standard conditions (T = 298.15 K, P = 100 kPa, concentration of 1 M).

Constant R = 8.314 J/(mol·K), T = (273.15 + °C) K, J is the reaction quotient.

For gases: $J = \frac{\{P(Y)/P^\theta\}^y\{P(Z)/P^\theta\}^z}{\{P(C)/P^\theta\}^c\{P(D)/P^\theta\}^d}$

For liquid: $J = \frac{\{c(Y)/c^\theta\}^y\{c(Z)/c^\theta\}^z}{\{c(C)/c^\theta\}^c\{c(D)/c^\theta\}^d}P^\theta = 100\ kPa,\ c^\theta = 1\ moL\cdot L^{-1}$

6.2. CO conversion to acetic acid and ethanol under different CO pressures and pH at 25, 33 and 55 °C

6.2.1. Acetic acid production

Gibbs free energy for the production of acetic acid from CO ($\Delta_rG_m(HAc)$) can be calculated according to Eq. (2) and Eq. (3). One mole acetic acid and accordingly 1 mol CO_2 are assumed to be produced (Eq. (2)). Considering acetic acid as a weak acid and the ionization constant (pKa) (Eqs. (4)–(7)), $\Delta_rG_m(HAc)$ is a function of CO gas pressure, CO_2 gas pressure and pH ($-lg[H^+]$) (Eq. (8)). CO_2 is a product along with acetic acid with a molar ratio of 1 and hence its concentration is 1 moL·L^{-1}. Therefore, the CO_2 gas pressure can be calculated according to the Ideal-Gas Equation (Eq. (9)). The final $\Delta_rG_m(HAc)$ is a function of CO gas pressure and pH (Eq. (10)).

Fig. 5 shows the Gibbs free energy of production of acetic acid from different CO gas pressures (0.01–10 bar) and pH varying from 1 to 12, at 25, 33 and 55 °C. Acetic acid production is enhanced with increased CO gas pressures and pH. This is in accordance with experimental data suggesting that acetic acid production was enhanced at higher pH than the pH for ethanol production (section 4.2.1). Increased CO gas pressures enable more CO to be dissolved in the liquid medium and thus enhanced the mass transfer. A pH decrease enhances the Gibbs free energy of acetic acid production at 25, 33 and 55 °C. However, the Gibbs free energy increase was slow when the pH was lower than 4.5 and kept relatively stable when the pH was lower than 4. Interestingly, autotrophic solventogenic *Clostridium* metabolism can also be inhibited when the pH is lower than 4.5 (Fast et al., 2015). At the same pH and CO gas pressure, acetic acid production is thermodynamically enhanced at 55 °C compared to 33 and 25 °C (Fig. 5). However, thermophilic ethanol producing microorganisms have been seldomly reported.

$$4CO + 2H_2O \rightarrow CH_3COO^- + H^+ + 2CO_2 \quad \Delta G^\theta = -154.6kJ/mol \tag{2}$$

$$\Delta_rG_m(HAc) = \Delta G_r^0 + RTln\frac{[CH_3COO^-][H^+][p_{CO_2}]^2}{[p_{CO}]^4} \tag{3}$$

$$CH_3COOH \rightarrow CH_3COO^- + H^+ \quad K_a = 1.76 \times 10^{-5} \tag{4}$$

$$K_a = \frac{[CH_3COO^-][H^+]}{CH_3COOH} \tag{5}$$

The total concentration of acetic acid is:

$$[Acid] = [CH_3COO^-] + [CH_3COOH] \tag{6}$$

From Eqs. (5) and (6),

$$[CH_3COO^-] = \frac{[Acid]K_a}{K_a + [H^+]} \tag{7}$$

Thus,

$$\Delta_rG_m(HAc) = \Delta G_r^0 + RTln\frac{[Acid][p_{CO_2}]^2}{[p_{CO}]^4} + RTln\frac{K_a[H^+]}{K_a + [H^+]} \tag{8}$$

There is production of 1 mol acetic acid and 2 mol CO_2,

$$The\ Ideal - Gas\ Equation\ is\ pV = nRT \tag{9}$$

With 22.4 L·mol^{-1}, p = nRT/V = [2*8.314*(273.15 + 33)/(22.4*0.001)] Pa = 227.3 kPa = 2.273 bar

p-Pa, V-m^3, n-mole, R = 8.314 J/(mol·K), T-K

$$\Delta G_r^{'} = \Delta G_r^0 + RTln\frac{1*2.273\hat{2}}{[p_{CO}]^4} + RTln\frac{K_a[H^+]}{K_a + [H^+]} \tag{10}$$

6.2.2. Ethanol production

Theoretically, ethanol can be produced from CO or acetic acid and CO (WLP). Therefore, the Gibbs free energy of production of ethanol

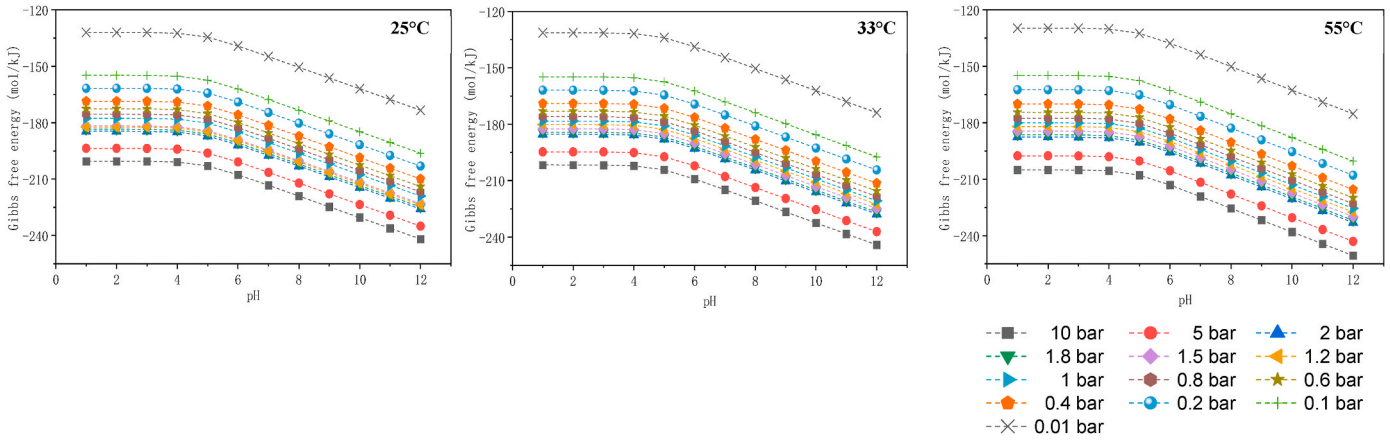

Fig. 5. The theoretical Gibbs free energy of CO to acetic acid when pH varied from 1 to 12, corresponding to different CO gas pressures (0.01–10 bar) at 25, 33 and 55 °C.

from CO ($\Delta_r G_m(EtOH)$) can be calculated in two different ways, according to Eq. (11) and Eq. (13).

1) $\Delta_r G_m(EtOH)$ from CO (Eq. (11))

According to Eq. (11), it is considered that CO is directly converted to ethanol and does not involve acetic acid. Thus, 1 mol ethanol and accordingly 4 mol CO_2 are assumed to be produced. Therefore, $\Delta_r G_m(EtOH)$ only depends on the CO gas pressure (Eq. (12)).

$$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2 \quad \Delta G^{\theta} = -271.4 kJ/mol \tag{11}$$

There is production of 1 mol ethanol and 4 mol CO_2.

The Ideal-Gas Equation, with 22.4 L·mol^{-1}, is pV = nRT, p = nRT/V = [4*8.314*(273.15 + 33)/(22.4*0.001)] Pa = 454.5 kPa = 4.545 bar

p-Pa, V-m^3, n-mole, R = 8.314 J/(mol·K), T-K

$$\Delta_r G_m(EtOH) = -271.4 + RTln\frac{1*4.545\hat{4}}{[p_{CO}]^6} \tag{12}$$

2) $\Delta_r G_m(EtOH)$ from CO and acetic acid

According to Eq. (13), it is considered that CO and acetic acid are converted to ethanol. Gibbs free energy of ethanol production is shown in Eq. (14). One mole ethanol and accordingly 2 mol CO_2 are assumed to be produced. Referring to section 6.2.1, $\Delta_r G_m(EtOH)$ is a function of CO gas pressure and pH (Eq. (15)).

$$2CO + CH_3COO^- + H^+ + H_2O \rightarrow CH_3CH_2OH + 2CO_2 \tag{13}$$

$$\Delta_r G_m(EtOH) = \Delta G_r^0 + RTln\frac{[CH_3CH_2OH][p_{CO_2}]^2}{[Acid][p_{CO}]^2} + RTln\frac{K_a + [H^+]}{K_a[H^+]} \tag{14}$$

$$\Delta_r G_m(EtOH) = \Delta G_r^0 + RTln\frac{1*2.273^2}{[Acid][p_{CO}]^2} + RTln\frac{K_a + [H^+]}{K_a[H^+]} \tag{15}$$

Fig. 6 shows the Gibbs free energy of production of ethanol from different CO gas pressures (0.01–10 bar) and pH values varying from 1 to 12, at 25, 33 and 55 °C. Fig. 6 shows increased CO gas pressures and lower temperatures, among 25, 33 and 55 °C, enhance thermodynamically ethanol production. This is in accordance with published experimental data that sub-mesophilic conditions (e.g. 25 °C) enhanced ethanol production compared to mesophilic conditions (37 °C) (Ramió-Pujol et al., 2015; He et al., 2021a). Besides, the decrease of pH enhances ethanol production (Fig. 6), which is in accordance with the experimental discussion highlighting that a low pH enhanced ethanol production (section 4.2.1).

6.3. CO to butyric acid and butanol under different CO pressures and pH, at 25, 33 and 55 °C

6.3.1. Butyric acid production

The Gibbs free energy for the production of butyric acid from CO ($\Delta_r G_m(HBu)$) can be calculated according to Eq. (16). One mole butyric acid and accordingly 6 mol CO_2 are assumed to be produced. $\Delta_r G_m(HBu)$ is a function of CO gas pressure (Eq. (17)). Then, butyric acid production is thermodynamically enhanced along with CO pressure increases and slightly enhanced at 25 °C compared to 33 and 55 °C (Fig. 7).

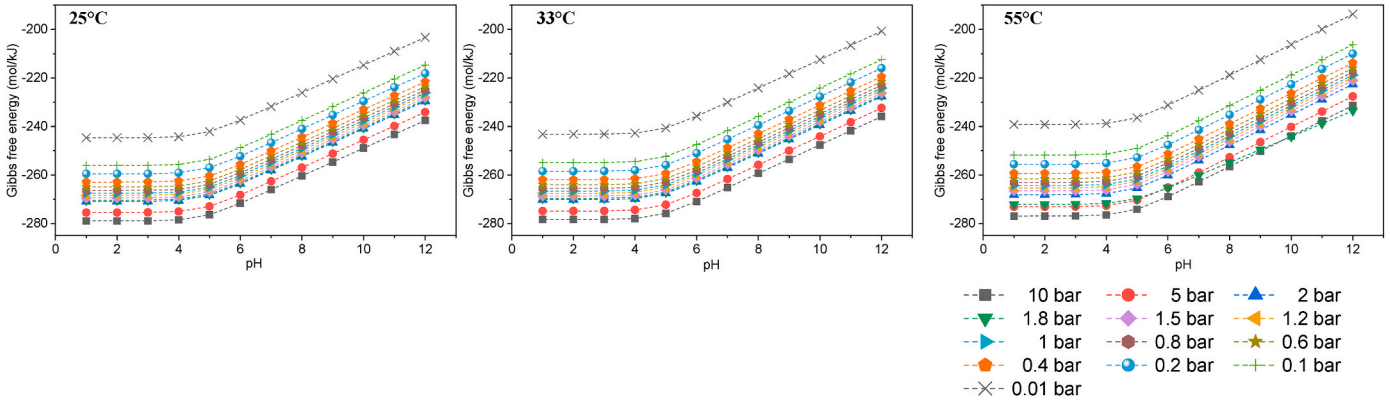

Fig. 6. The theoretical Gibbs free energy of CO to ethanol conversion under different CO gas pressures (0.01–10 bar) at 25, 33 and 55 °C (Calculated for 1 mol ethanol produced).

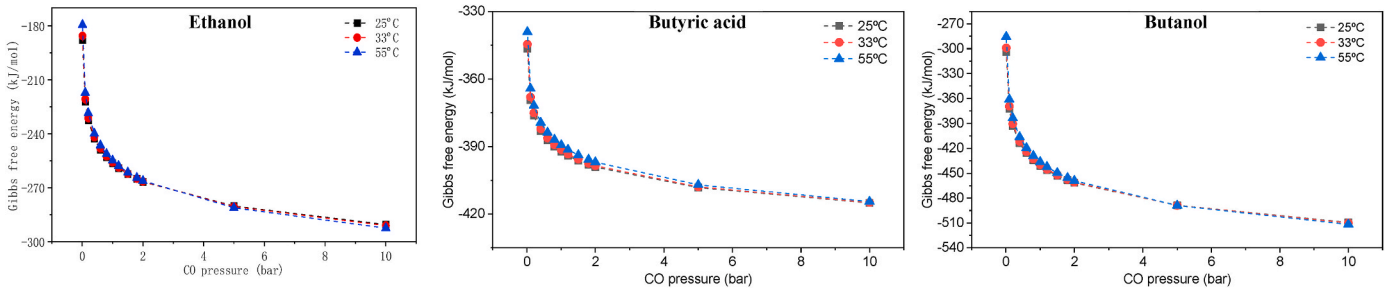

Fig. 7. The theoretical Gibbs free energy of CO to ethanol, butyric acid and butanol conversion under different CO gas pressures (0.01–10 bar) at 25, 33 and 55 °C (Calculated for 1 mol butyric acid production and without considering pH).

$$10CO + 4H_2O \rightarrow CH_3CH_2CH_2COOH + 6CO_2 \quad \Delta G^{\theta} = -420.8 kJ/mol \tag{16}$$

There is production of 1 mol butyric acid and 6 mol CO_2.

The Ideal-Gas Equation, with 22.4 L·mol^{-1}, is pV = nRT,

p = nRT/V = [6*8.314*(273.15 + 33)/(22.4*0.001)] Pa = 6.819 kPa = 6.819 bar

$$\Delta_r G_m(HBu) = -420.8 + RTln\frac{1*6.819^6}{[p_{CO}]^4} \tag{17}$$

6.3.2. Butanol production

The Gibbs free energy of production of butanol from CO ($\Delta_r G_m(BtOH)$) can be calculated according to Eq. (18). One mole butanol and accordingly 8 mol CO_2 are assumed to be produced. $\Delta_r G_m(BtOH)$ is a function of CO gas pressure (Eq. (19)). Then, butanol production is thermodynamically enhanced along with CO pressure increases and slightly enhanced at 25 °C compared to 33 and 55 °C (Fig. 7). However, when the gas pressure increases above 2 bar, the decrease extent of Gibbs free energy for butyric acid production becomes slow. The suggested CO gas pressure would hence be around 2 bar instead of higher gas pressures, which could also reduce the cost to pressurize gases in full scale applications.

$$12CO + 5H_2O \rightarrow CH_3CH_2CH_2CH_2OH + 8CO_2 \quad \Delta G^{\theta} = -486.4 kJ/mol \tag{18}$$

There is production of 1 mol butanol and 8 mol CO_2.

The Ideal-Gas Equation, with 22.4 L·mol^{-1}, is pV = nRT,

p = nRT/V = [8*8.314*(273.15 + 33)/(22.4*0.001)] Pa = 9.092 kPa = 9.092 bar

$$\Delta_r G_m(BtOH) = -486.4 + RTln\frac{1*9.902\hat{8}}{[p_{CO}]^{12}} \tag{19}$$

7. Concluding remarks and future perspectives

This study reviewed process parameters involved in solventogenic syngas bioconversion by pure and mixed defined and undefined cultures. CO gas pressure increase, pH decrease and mesophilic and sub-mesophilic temperatures enhance solventogenesis, which has also been demonstrated and supported through thermodynamic calculations. Trace metals (such as W and Mo) and a proper nitrogen source (such as 0.5 g/L yeast extract) also favor solventogenic ethanol production. Adding adsorptive and conductive materials (such as nanoparticles, activated carbon and biochar) as well as electron shuttles (such as methyl viologen) has been used for enhanced mass transfer and biomass growth. Besides, longer carbon chain solvents production can be obtained via mixotrophy or exogenous and endogenous acid addition in mixed cultures.

The thermodynamic calculations provide a novel approach for understanding and evaluating the effect of CO gas pressure and pH for enhanced ethanol and butanol production from syngas under thermophilic, mesophilic and sub-mesophilic conditions. However, biomass growth and its reduction ability were not considered, which also limits the application of the thermodynamic calculations in real-case conditions. Future studies on thermodynamics should consider real-case conditions, and expand them with syngas composition, biomass concentration and mass transfer parameters.

The mechanisms of pH on solventogenesis and metabolic processes of microorganisms remains to be further studied. Considering the various parameters affecting the process, the exploration of the combination and optimization of different parameters to maximize alcohols production should be considered. Model simulations could be an effective way to provide insights into parameter optimization. The development of microbial analysis technologies such as metagenomic sequencing, molecular analysis and proteomics will also enable better understanding of the involvement of specific enzyme and gene level aspects allowing to select efficient solventogenic strains via gene modification.

Several trials have demonstrated the possibility to efficiently produce longer chain alcohols such as butanol and hexanol, among others via mixotrophy and with mixed cultures. However, future studies should be developed to investigate the metabolic pathway and mechanisms behind mixed culture processes, such as how interspecies communication occurs, via ^{13}C labeled syngas combined with nuclear magnetic resonance (NMR) analyses. On the other hand, finding cheap and economic feedstock sources remains an issue to be solved in order to improve economic aspects of syngas bioconversion. Syngas bioconversion for biofuels production has been deemed as third generation biofuels, with merits of utilizing a wide range of feedstocks for gasification such as agriculture wastes and the organic fraction of municipal solid wastes. On the other hand, it helps in reaching carbon neutral emissions together with valuable chemicals production.

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Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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