Use of Syngas for the Production of Organic Molecules by Fermentation

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1 Introduction

Exploring environment-friendly methods, such as anaerobic fermentation, to convert biodegradable organic matter into biofuels and chemicals have drawn worldwide interest (Henstra et al. 2007; Latif et al. 2014; Miltner et al. 2010). However, direct conversion of recalcitrant organic wastes by biological processes entails difficulty, and a significant amount of non-biodegradable materials remains in effluents. Most biodegradable cellulose (40–50%) and hemicellulose (20–40%) materials in the biomass are packed with lignin (10–40%), which is resistant to microbial degradation (Abubackar et al. 2011; Meng and Ragauskas 2014; Zeng et al. 2014). Gasification, a thermochemical process, can convert mineral fuels or biomass into synthesis gas (syngas) as a mixture of CO, H_2, and minor components CO_2, CH_4, H_2S, and NO_x (Fabbri and Torri 2016; Latif et al. 2014; Shen et al. 2015). Syngas as a type of cleaning chemical feedstock can be further used for production by both chemical methods (e.g., Fischer–Tropsch synthesis) and biotechnological methods (e.g., syngas fermentation) (Latif et al. 2014; Shen et al. 2015).

As an important biotechnological technique, syngas fermentation provides lower operational temperature, lower pressure, as well as higher selectivity and resistance to toxicity that those of Fischer–Tropsch synthesis (Bengelsdorf et al. 2013; Ganigué et al. 2016; Liew et al. 2016; Massaro et al. 2015). Consequently, it provides a potential pathway to use hardly biodegradable organic materials, such as lignocellulose and sludge, for the production of biofuel and volatile fatty acids (VFAs)

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(Henstra et al. 2007; Jing et al. 2017; Latif et al. 2014; Liew et al. 2016; Zhang et al. 2013b). Thus far, syngas fermentation focuses on pure culture and co-culture under mesophilic conditions and is proposed to convert syngas to VFAs (such as acetate and butyrate), ethanol, butanol, and/or caproate via microbes, such as *Clostridium ljungdahlii*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, and *Alkalibaculum bacchi* (Liew et al. 2016; Martin et al. 2016; Ramió-Pujol et al. 2015; Schuchmann and Muller 2014). Compared with mixed culture fermentation (MCF), pure culture or co-culture fermentation is typically challenged by strain degeneration and contamination (Esquivel-Elizondo et al. 2017; Henstra et al. 2007). CO toxicity to some bacteria also impedes CO conversion (Esquivel-Elizondo et al. 2017; Jing et al. 2017). Bertsch and Müller (2015) demonstrated that the hydrogen-dependent CO_2 reductase of *Acetobacterium woodii* is highly sensitive to CO, consequently impeding the growth of *A. woodii* on CO as a sole carbon and energy source. Thus, mixed culture syngas fermentation can potentially facilitate the simultaneous conversion of H_2 and CO by different enriched bacteria.

In syngas MCF, the functional bacteria are acetogenic bacteria, such as *C. ljungdahlii*, *C. autoethanogenum*, and *C. carboxidivorans*. These bacteria can convert CO, H_2, and CO_2 to acetate, ethanol, and other products via the Wood–Ljungdahl pathway (Köpke et al. 2011), as shown in Fig. 1. Other bacteria, such as *Clostridium kluyveri*, can produce longer carbon-chain metabolites, including butyrate, caproate, and caprylate from ethanol and acetate via reverse β-oxidation reaction (Fig. 1) (Seedorf et al. 2008). When the methanogens archaea are enriched in the reactor, the produced metabolites and syngas are also consumed to produce methane. Biochemical reactions in syngas fermentation are also thermodynamically controlled. Thus, the basic bioreactions and thermodynamics are summarized in Sect. 2.

The operating conditions—pH, temperature, CO and H_2 partial pressure, and impurities of tar and NO_x—potentially induce changes in the microbial community or metabolic pathway in mixed culture fermentation. These factors are reviewed in Sect. 3. Meanwhile, the low solubility of H_2 and CO in the water phase also limits syngas utilization (Henstra et al. 2007). The configurations of the reactor, such as the continuous stirred-tank reactor (CSTR), trick biofilm reactor, and hollow fiber membrane biofilm reactor (HfMBR), are summarized in Sect. 3.4.

Lastly, syngas pretreatment was generally disregarded in syngas fermentation, which was demonstrated to reduce bacterial activity; thus, these technologies were indispensable and should be coupled with syngas fermentation (Benalcázar et al. 2017; Sheth and Babu 2010). On the other hand, the inhibition of organic acids, particularly at acidic pH, presents a main challenge for bacteria because the inward diffusion of organic acids over the cytoplasmic membrane leads to the dissipation of the proton-motive force, and bacteria have to transport these metabolites by energy consumption in the form of ATP (Louis et al. 2004; Zhang et al. 2013c). Meanwhile, the accumulation of ethanol causes the hyperpolarization of the bacterial lipid bilayer, which consequently decreases membrane integrity and inhibits bacterial activity (Thammasittirong et al. 2013). Thus, coupling syngas fermentation with other technologies, such as syngas pretreatments and membrane technology, is necessary for its

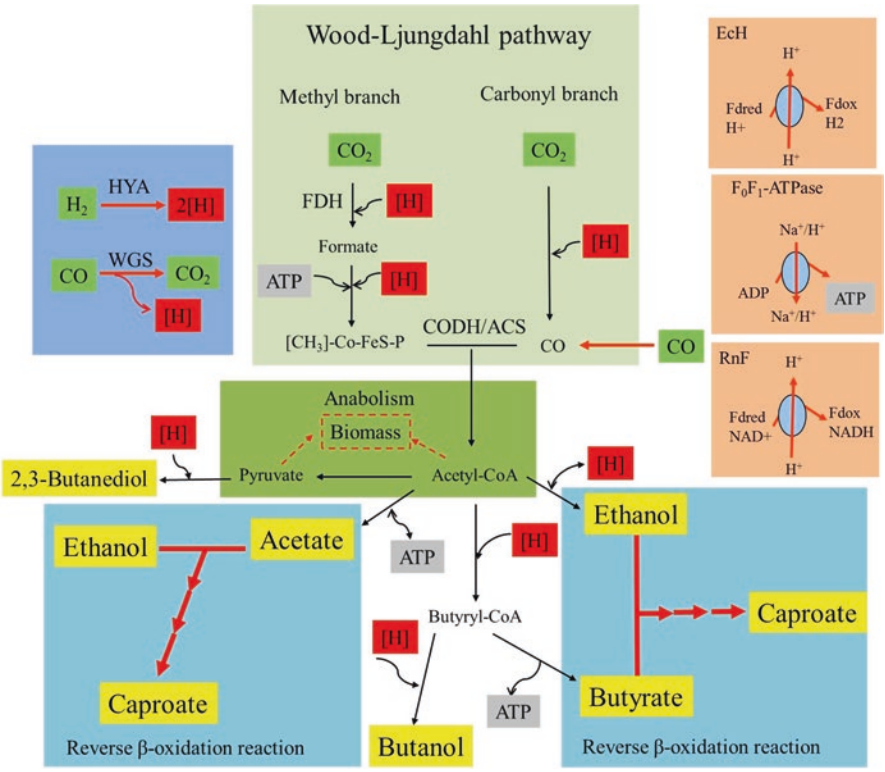

Fig. 1 Metabolic pathways in syngas fermentation (Diender et al. 2015; Schuchmann and Muller 2014; Seedorf et al. 2008)

application (Dai et al. 2017; Liu and Qureshi 2009). Such application is summarized in Sect. 4. Other promising technologies, such as PHA production and microbial fuel cells (MFCs), are also reviewed. Thus, this chapter is expected to promote the development and worldwide application of syngas fermentation in the future.

2 Bioreactions and Thermodynamics in Syngas Fermentation

The metabolic pathways of syngas fermentation are presented in Fig. 1. Energy conservation occurs by substrate-level phosphorylation in a catabolic reaction, ion-motive force, and energy conservation via electron bifurcation reaction, which involves key enzymes such as EcH (e.g., that in *Moorella thermoacetica*), Rnf complex (e.g., that in *C. ljungdahlii*), and ATPase (Angenent et al. 2016; Basen and Müller 2017; Diender et al. 2015; Drake et al. 2008; Schuchmann and Muller 2014; Seedorf et al. 2008). The main metabolites are identified as acetate, butyrate, caproate, and ethanol (Bengelsdorf et al. 2013; Diender et al. 2015; Spirito et al. 2014).

Hydrogen is initially converted by hydrogenase to reducing equivalents, and CO can be transformed to CO_2 and reducing equivalents in biological water–gas shift reactions [such as that in *C. autoethanogenum* (Liew et al. 2016)], as shown in Fig. 1. The Wood–Ljungdahl pathway consists of two separate branches—the carbonyl branch and the methyl branch—in acetogens such as *C. ljungdahlii* (Köpke et al. 2010; Muller 2003). In the carbonyl branch, CO_2 is reduced to CO via a bifunctional enzyme of the carbon monoxide dehydrogenase/acetyl–CoA synthase (CODH/ACS). In the methyl branch, CO_2 is reduced to formate via formate dehydrogenase, which is finally converted to $[CH_3]$–Co–FeS–P. The bifunctional enzyme (CODH/ACS) fuses CO with both the produced methyl group and CoA to form acetyl–CoA. Acetyl–CoA is the important intercellular intermediate, which can be converted to pyruvate, acetate, ethanol, butyrate, and so on via different functional enzymes and is the building block for biomass production in anabolism. The bioreactions for acetate and ethanol production from syngas are as follows:

- Acetate production from CO

$$4CO + 2H_2O \rightarrow C_2H_3O_2^- + H^+ + 2CO_2 \tag{1}$$

- Ethanol production from CO

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2 \tag{2}$$

- Acetate production from H_2 and CO_2

$$4H_2 + 2CO_2 \rightarrow C_2H_3O_2^- + H^+ + 2H_2O \tag{3}$$

- Ethanol production from H_2 and CO_2

$$6H_2 + 2CO_2 \rightarrow C_2H_5OH + 3H_2O \tag{4}$$

The produced acetate, butyrate, and ethanol in the Wood–Ljungdahl pathway are chemical building blocks for the production of longer carbon-chain molecules, such as caproate, via reverse β-oxidation reaction in *C. kluyveri* (Seedorf et al. 2008; Spirito et al. 2014) in which electron bifurcation and two membrane-associated, energy-converting enzyme complexes involved in fermentation, ferredoxin:NAD oxidoreductase and ATP synthase, provide the energy source. The bioreactions for caproate production from acetate, butyrate, and ethanol are as follows:

- Caproate production from acetate and ethanol

$$C_2H_3O_2^- + 2C_2H_5OH \rightarrow C_6H_{11}O_2^- + 2H_2O \tag{5}$$

- Caproate production from butyrate and ethanol

$$C_4H_7O_2^- + C_2H_5OH \rightarrow C_6H_{11}O_2^- + H_2O \tag{6}$$

Finally, methanogens can convert acetate and H_2/CO_2 to CH_4, referred to as aceticlastic methanogenesis and hydrogenotrophic methanogenesis, respectively (Dai et al. 2017; Thauer et al. 2008). The former (Eq. 7) is conducted by *Methanosarcinaceae* and *Methanosaetaceae*, whereas the latter (Eq. 8) is performed by *Methanomicrobiales* and *Methanobacteriales* (Karakashev et al. 2006).

$$C_2H_3O_2^- + H^+ \rightarrow CH_4 + CO_2 \tag{7}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{8}$$

The biochemical reactions in Fig. 1 are generally constrained by thermodynamic control (Richter et al. 2016; Schuchmann and Muller 2014). The standard Gibbs free energy of formation and standard enthalpy of formation for the relevant compounds are shown in Table 1 (Speight 2005; Thauer et al. 1977). The detailed calculation of the reaction of Gibbs free energy is provided elsewhere (Bastidas-Oyanedel et al. 2008; Kleerebezem and Van Loosdrecht 2010; Lee et al. 2008).

3 Influencing Factors in Syngas Fermentation

Operating conditions, such as pH, temperature, and CO and H_2 partial pressure, potentially trigger changes in microbial community composition and/or metabolic flow in mixed culture fermentation, consequently affecting the performances of the reactors, as summarized in Table 2.

Table 1 ΔG_f^0 and ΔH_f^0 of syngas fermentation metabolites

Metabolite	State	ΔG_f^0 (kJ/mol)	ΔH_f^0 (kJ/mol)
CO	Gas	−137.16	−110.53
H_2	Gas	0	0
CO_2	Gas	−394.36	−393.50
Acetate	Aqueous	−369.31	−486.01
Ethanol	Aqueous	−181.64	−288.3
Butyrate	Aqueous	−352.63	−535.55
Butanol	Aqueous	−162.5	−327.3
2,3-Butanediol	Aqueous	−322.0	−541.5
Caproate	Aqueous	−336.0	–
Hexanol	Aqueous	−152.3	−377.5
H_2O	Liquid	−237.19	−285.83

3.1 Effect of Temperature

Temperature can shift the dominant bacteria or the main metabolic pathways and play an important role in syngas MCF. Using H_2/CO_2 as the substrates in HfMBR, Zhang et al. found a mixture of acetate (7.4 g/L), butyrate (1.8 g/L), caproate (0.98 g/L), and caprylate (0.42 g/L) that was accumulated at pH 6.0 and in 35 °C syngas MCF, where *Clostridium* spp. (such as *C. ljungdahlii* and *C. kluyveri*) as the dominant bacteria (Zhang et al. 2013b). Wang et al. (2017) demonstrated that with an increase in temperature to 55 °C, *Thermoanaerobacterium* (66%) became the main bacterium and acetate comprised more than 98.5% and 99.1% of total metabolites in batch and continuous modes, respectively. Owing to a decrease in the diffusion coefficients of acidic metabolites at low temperature, metabolite inhibition weakened (Ramió-Pujol et al. 2015; Zhang et al. 2013c). As temperature decreased to 25 °C, acetate, ethanol, butyrate, and caproate were the main metabolites, as determined in the study by Wang et al. (2018b). Caproate concentration (5.7 g/L) was particularly higher than that of pure culture fermentation (*C. carboxidivorans* P7, 1.05 g/L).

Ramió-Pujol et al. (2015) used H_2/CO as the substrate and compared the metabolites in pure culture fermentation of *C. carboxidivorans* P7 at 25 and 35 °C; acetate (1.6 g/L) was found to be the main metabolite, with apparent accumulation of caproate (1.05 g/L) at 25 °C. Meanwhile, as temperature increased to 37 °C, no caproate was produced. Under thermophilic conditions (55 °C), the dominant bacteria in syngas MCF were *Desulfotomaculum* and *Caloribacterium*, and the main product was acetate (0.15 g/L), as determined in the study by Alves et al. (2013). We recently compared metabolite distribution in HfMBR by using CO and H_2 as the substrate; acetate (4.22 g/L), butyrate (1.35 g/L), caproate (0.88 g/L), and caprylate (0.52 g/L) were detected at 35 °C, whereas acetate (24.6 g/L) and minimal butyrate (less than 1 g/L) were detected at 55 °C (unpublished data).

In addition, the changes in Gibbs free energy ($\Delta G'$) of the main bioreactions in syngas (CO/H_2) fermentation under standard conditions, except for pH at 7.0, are shown in Table 3. Except for caproate production, $\Delta G'$ is higher at a low temperature of 25 °C than at 35 °C and 55 °C; thus, more energy can be used for biomass growth or maintenance at low temperature from the viewpoint of thermodynamics. Ramió-Pujol et al. (2015) determined that the maximum OD600 values of *C. carboxidivorans* P7 at 25 °C (OD600, 1.2) was higher than that at 35 °C (OD600, 0.55). On the other hand, all $\Delta G'$ values of acetate and ethanol production from CO (Eqs. 1 and 2) were lower than those from CO_2 and H_2 (Eqs. 3 and 4), allowing bacteria to obtain more energy from CO utilization. Although a high temperature favors caproate production from the viewpoint of thermodynamics, no caproate has been detected in the thermophilic reactor. As a known caproate production bacterium, *C. kluyveri* only lives under mesophilic conditions (Seedorf et al. 2008; Thauer et al. 1968). Thus, the enriched bacteria are also considered a critical factor for the determination of metabolite production.

Table 2 Operating conditions and metabolites in syngas fermentation

Type of fermentation	Reactor configuration	Syngas composition	Temp. (°C)	pH	Main metabolites	References
Pure culture (*Clostridium carboxidivorans*)	Batch	CO (20%), CO_2 (15%), H_2 (5%), N_2 (60%), minor NO (200 ppm)	37	5.7	Ethanol (0.042 g/L)	Ahmed and Lewis (2007)
Pure culture (*Clostridium carboxidivorans*)	External HFM reactor	CO (20%), H_2 (5%), CO_2 (15%), N_2 (60%)	37	4.5–6.0	Acetate (5.0 g/L), ethanol (23.93 g/L)	Shen et al. (2014)
Pure culture (*Clostridium carboxidivorans*)	Batch	CO (32%), H_2 (32%), N_2 (28%), CO_2 (8%)	25 and 37	4.8–5.9	25 °C: acetate (1.6 g/L), caproate (1.05 g/L); 37 °C: acetate (3.5 g/L), butyrate (0.36 g/L)	Ramió-Pujol et al. (2015)
Pure culture (*Clostridium autoethanogenum*)	Batch	CO (100%)	35	4.75	Ethanol (0.87 g/L)	Abubackar et al. (2015)
Pure culture (*Clostridium autoethanogenum*)	Continuous	CO (100%)	35	6.0	Acetate (0.91 g/L), ethanol (0.91 g/L)	Abubackar et al. (2015)
Pure culture (*Clostridium ragsdalei*)	Trickle-bed reactor	CO (38%), CO_2 (28.5%), H_2 (28.5%) N_2 (5%)	37	4.6	Ethanol (5.7 g/L), acetate (12.3 g/L)	Devarapalli et al. (2016)
Pure culture (*Alkalibaculum bacchi*)	Batch	CO (40%), CO_2 (30%), H_2 (30%)	37	8.0	Propanol (0.4 g/L), butanol (0.5 g/L), hexanol (0.8 g/L)	Liu et al. (2014)
Co-culture (*Alkalibaculum bacchi* and *Clostridium propionicum*)	Batch	CO (40%), CO_2 (30%), H_2 (30%)	37	8.0	Propanol (1.0 g/L), butanol (0.8 g/L), hexanol (1.0 g/L)	Liu et al. (2014)
Mixed cultures	Batch	CO (20%), CO_2 (15%), H_2 (20%), CH_4 (3%), N_2 (42%)	37	6.0	Ethanol (2.2 g/L), acetate (0.9 g/L)	Singla et al. (2014)
Mixed culture	Batch	H_2 (1.5 bar)	35	6.0	Ethanol (0.17 g/L), propanol (0.48 g/L), butanol (0.27 g/L)	Steinbusch et al. (2008)
Mixed culture	Batch	CO (100%)	55	7.0	Acetate (0.15 g/L)	Alves et al. (2013)
Mixed culture	Batch	H_2 (32%), CO (32%), CO_2 (8%), N_2(28%)	37	4.8	Ethanol (1.7 g/L), butanol (1.1 g/L), hexanol (0.6 g/L)	Ganigué et al. (2016)

(continued)

Table 2 (continued)

Type of fermentation	Reactor configuration	Syngas composition	Temp. (°C)	pH	Main metabolites	References
Mixed culture	CSTR	CO (55%), H_2 (20%), CO_2 (10%), Ar (15%)	37	7.0	Ethanol (6.50 g/L), acetate (5.43 g/L)	Mohammadi et al. (2012)
Mixed culture	HfMBR	H_2 (60%) and CO_2 (40%)	35	6.0	Acetate (7.4 g/L), butyrate (1.8 g/L), caproate (0.98 g/L), caprylate (0.42 g/L)	Zhang et al. (2013b)
Mixed culture	HfMBR	H_2 (60%) and CO_2 (40%)	35	4.5	Batch: acetate (12.5 g/L) Continuous: acetate (0.4 g/L/d)	Zhang et al. (2013a)
Mixed culture	HfMBR	H_2 (60%) and CO_2 (40%)	25	6.0	Acetate (31.1 g/L), butyrate (4.1 g/L), caproate (5.7 g/L)	Wang et al. (2018b)
Mixed culture	HfMBR	H_2 (60%) and CO_2 (40%)	55	6.0	Batch: acetate (42.4 g/L) Continuous: acetate (10.5 g/L/d)	Wang et al. (2017)
Mixed culture	HfMBR	CO (60%) and H_2 (40%)	35	4.5	Ethanol (16.9 g/L)	Wang et al. (2018a)

3.2 *Effect of pH*

The inward diffusion of organic acids over the cytoplasmic membrane at acidic pH presents major challenge for bacteria because it leads to energy dissipation (Louis et al. 2004; Zhang et al. 2013c). Wilbanks and Trinh (2017) recently found that higher concentrations and/or hydrophobicity of metabolites cause the increased growth inhibition of *E. coli*. Consequently, acidic pH was generally considered a main factor for the shifting of metabolites to produce alcohol (Datar et al. 2004; Fernández-Naveira et al. 2017; Valgepea et al. 2017). Abubackar et al. (2015) reported that at pH 4.75, no acetate was produced, and ethanol concentration reached a maximum of 0.87 g/L; at pH of 6.0, almost equal amounts of ethanol and acetate were formed from CO, obtaining 0.91 g/L. Ganigué et al. (2016) indicated that at pH of about 4.8 in the batch mode, a mixture of ethanol (1.7 g/L), butanol (1.1 g/L), and hexanol (0.6 g/L) was produced from syngas (32% H_2, 32% CO, 8% CO_2, and 28% N_2). Liu et al. (2014) demonstrated that compared with the *A. bacchi* strain CP15 monoculture (propanol of 0.4 g/L, butanol of 0.5 g/L, and hexanol of 0.8 g/L), the addition of propionic acid, butyric acid, and hexanoic acid to the mixed culture of CP15 and *Clostridium propionicum* resulted in a 50% higher conversion efficiency of these acids to their respective alcohols (propanol of 1.0 g/L, butanol of 0.8 g/L, and hexanol of 1.0 g/L). Singla et al. (2014) enriched several mixed cultures and optimized their growth conditions for ethanol production, obtaining a maximum ethanol concentration of 2.2 g/L.

Using H_2/CO_2 as the substrate, Zhang et al. (2013b) found a mixture of acetate (7.4 g/L), butyrate (1.8 g/L), caproate (0.98 g/L), and caprylate (0.42 g/L). The mixture was accumulated at pH 6.0 and temperature of 35 °C in syngas MCF, with *Clostridium* spp. (such as *C. ljungdahlii* and *C. kluyveri*) as the dominant bacteria; meanwhile, as pH was reduced to 4.0, the metabolite only consisted of acetate (12.5 g/L), and the dominant bacteria were identified as *C. ljungdahlii* and *C. drakei*

Table 3 Change in Gibbs free energy of main bioreactions in syngas (CO/H_2) fermentation under standard conditions

Bioreactions	$\Delta G'$ (kJ/mol) *		
	25 °C	35 °C	55 °C
Acetate production from CO: $4CO + 2H_2O \rightarrow C_2H_3O_2^- + H^+ + 2CO_2$ (1)	−175.0	−172.2	−166.6
Ethanol production from CO: $6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$ (2)	−224.5	−220.6	−212.9
Acetate production from CO_2 and H_2: $4H_2 + 2CO_2 \rightarrow C_2H_3O_2^- + H^+ + 2H_2O$ (3)	−95.0	−87.8	−77.4
Ethanol production from CO_2 and H_2: $6H_2 + 2CO_2 \rightarrow C_2H_5OH + 3H_2O$ (4)	−104.5	−96.0	−79.1
Caproate: $C_2H_3O_2^- + 2C_2H_5OH \rightarrow C_6H_{11}O_2^- + 2H_2O$ (5)	−77.7	−81.5	−89.1
Caproate: $C_4H_7O_2^- + C_2H_5OH \rightarrow C_6H_{11}O_2^- + H_2O$ (6)	−38.9	−43.0	−51.3

(Zhang et al. 2013a). Thus, apart from acidic pH, CO was also considered a main factor promoting ethanol production. However, the accumulation of ethanol leads to the hyperpolarization of the bacterial lipid bilayer, which consequently decreases membrane integrity and inhibits bacterial activity (Thammasittirong et al. 2013). Thus, removing the accumulation of organic acids and ethanol from the bulk solution could also increase bacterial activity.

The Gibbs free energy ($\Delta G'$) of acetate and ethanol was calculated. The results are listed in Table 4. $\Delta G'$ of acetate (Eq. 1) and ethanol (Eq. 2) production from CO is higher than that from H_2 (Eqs. 3 and 4); thus, CO is the more suitable substrate for syngas fermentation (Diender et al. 2015). On the other hand, at neutral pH, $\Delta G'$ of ethanol production from CO (Eq. 2) is −220.6 kJ/mol and that from H_2 (Eq. 4) is −96.0 kJ/mol. Both values are higher than the values obtained for acetate production (−172.2 and −89.2 kJ/mol); thus, under neutral pH, bacteria can obtain more energy from CO utilization from the viewpoint of thermodynamics.

3.3 CO and H_2 Partial Pressure

CO and H_2 can inhibit hydrogenase activity and change the ratio of intercellular redox couplers, such as Fdred/Fdox and NADH/NAD^+, and consequently shift the metabolite distribution (Abubackar et al. 2015; Sancho-Navarro et al. 2016; Zhang et al. 2013c). Several studies demonstrated that hydrogen partial pressure (P_{H_2}) and CO partial pressure (P_{CO}) as factors could shift the dominant bacteria and change the metabolite distribution in syngas MCF (Peintner et al. 2010; Steinbusch et al. 2008; Temudo et al. 2008; Zhang et al. 2013a, b). Steinbusch et al. (2008) indicated that VFAs such as acetic, propionic, and butyric acids were reduced at P_{H_2} of 1.5 bar by MCF: the final alcohol concentrations were ethanol (0.17 g/L), propanol (0.48 g/L), and *n*-butanol (0.27 g/L). Bertsch and Müller (2015) revealed that the hydrogen-dependent CO_2 reductase of *A. woodii* was highly sensitive to CO; consequently, *A. woodii* failed to grow on CO as a sole carbon and energy source.

Table 4 Gibbs free energy ($\Delta G'$) of acetate and ethanol production at acidic pH in syngas (H_2 and CO) fermentation

Bioreactions	$\Delta G'$ (kJ/mol)[a]		
	pH 7.0	pH 6.0	pH 4.5
Acetate production from CO: $4CO + 2H_2O \rightarrow C_2H_3O_2^- + H^+ + 2CO_2$ (1)	−172.2	−166.3	−157.5
Ethanol production from CO: $6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$ (2)	−220.6	−220.6	−220.6
Homoacetogenesis: $4H_2 + 2CO_2 \rightarrow C_2H_3O_2^- + H^+ + 2H_2O$ (3)	−89.2	−83.3	−74.4
Ethanol production from H_2: $6H_2 + 2CO_2 \rightarrow C_2H_5OH + 3H_2O$ (4)	−96.0	−96.0	−96.0

[a]All calculated under standard conditions, except for 35 °C and acidic pH

Sancho-Navarro et al. (2016) recently analyzed the methane production pathway from syngas and determined that acetoclastic methanogens were the most sensitive to CO and that high CO concentrations led to a shift in the archaeal population to hydrogen-utilizing methanogens.

3.4 Reactor Configurations

Although syngas fermentation provides a platform for organic waste utilization, the poor aqueous solubility of H_2 and CO is a major limiting factor in syngas fermentation (Esquivel-Elizondo et al. 2017; Lee et al. 2016). Increasing the speed of the impeller (500 rpm in the study by Mohammadi et al. (2012)) in CSTR can provide high gas/liquid mass transfer coefficients with an agitation mechanism that allows the breakdown of large bubbles into smaller ones and improves gas–liquid mass transfer (Fernández-Naveira et al. 2017; Mohammadi et al. 2012). Mohammadi et al. (2012) operated a mesophilic (37 °C) CSTR with an agitation rate of 500 rpm and a working volume of 2 L; the produced metabolites were ethanol (6.50 g/L) and acetate (5.43 g/L). However, high agitation rates can also lead to high-power consumption and may inhibit bacterial activity (Henstra et al. 2007; Yasin et al. 2015; Zhao et al. 2014). The trickle-bed reactor was also proposed to resolve poor solubility; Devarapalli et al. (2016) proposed ethanol production in a semi-continuous trickle-bed reactor and found that the biofilm facilitates syngas utilization; the final ethanol and acetate concentrations were 5.7 and 12.3 g/L, respectively.

Increasing the specific gas–liquid interfacial area by membrane technologies can diminish the poor gas solubility (Henstra et al. 2007; Nerenberg 2016; Zhang et al. 2013b). Shen et al. (2014) found that the volumetric mass transfer coefficients (K_{La}) of the hollow fiber membrane were higher than those of most reactor configurations, such as CSTR and bubble columns. Zhang et al. (2013b) proposed a mesophilic HfMBR for the in situ consumption of H_2 and CO_2 with 100% utilization of H_2, with *Clostridium* spp. (such as *C. ljungdahlii* and *C. kluyveri*) as the dominant bacteria. In addition, the product contained a mixture of acetate (7.4 g/L), butyrate (1.8 g/L), caproate (0.98 g/L), and caprylate (0.42 g/L). In a thermophilic HfMBR (55 °C), acetate comprised more than 99% of total VFAs from H_2 and CO_2 MCF, but no caproate was produced (Wang et al. 2017). HfMBR also provides several advantages, such as low energy consumption and small reactor footprints (Martin and Nerenberg 2012). Moreover, the biofilm formed on the outer surface of the hollow fiber membrane may enhance bacterial resistance to CO toxicity. Jiang et al. (2011) reported that the butyric acid tolerance of *Clostridium tyrobutyricum* increased markedly after being immobilized in a fibrous-bed bioreactor and the final butyric acid concentration reached 86.9 g/L.

However, membrane fouling has been recognized as a key factor for lower running efficiency, higher operating cost, and shorter membrane lifespan (Drews 2010; Meng et al. 2017; Wang et al. 2014). Ayala et al. (2011) assigned a linear trend between membrane permeability loss (due to membrane fouling and cleaning) and operation

time, which indicated the recovered membrane permeability to reach a threshold minimum value for virgin membrane after about 7 years of operation. In HfMBR, a sufficient quantity of microorganisms attached to the membrane surface is necessary for efficient and stable operation; however, the smaller the size of the membrane pores, the higher the gas pressure and energy consumption (Munasinghe and Khanal 2010). Consequently, the energy problem still needs to be evaluated in future studies.

3.5 Impurities of Synthesis Gas

Syngas fermentation using artificial syngas formulated only with CO and H_2 remains the focus of research, whereas impurities such as NO_x are rarely studied (Benalcázar et al. 2017; Liew et al. 2016; Xu et al. 2011). Syngas is produced by thermochemical gasification; thus, minor components such as NO_x and ammonia can also potentially affect syngas fermentation (Benalcázar et al. 2017; Sheth and Babu 2010). Datar et al. (2004) found that in *C. carboxidivorans* $P7^T$ fermentation, cell growth stopped (with negligible death) when syngas directly produced from switchgrass was used as feedstock because the components of the original syngas might inhibit the hydrogenase enzyme. Ahmed and Lewis (2007) analyzed NO toxicity on the hydrogenase of *C. carboxidivorans* $P7^T$ and concluded that when NO content was below 40 ppm, inhibition could be tolerated by cells in a syngas fermentation system without compromising hydrogenase activity, cell growth, and product distribution. However, when the NO content was 200 ppm, hydrogenase activity remained completely inhibited, and ethanol concentration was only 0.042 g/L (Ahmed and Lewis 2007). Xu et al. (2011) indicated that the entrained tar particulates (above 0.025 mm), nitric oxide (0.004 mol%), and ammonia (above 0.25 mol/L) negatively affected the syngas fermentation process.

Except for NO_x, other impurities such as cyanide may also lower the performance of syngas fermentation. Benalcázar et al. (2017) recently reported that when lignocellulosic biomass and municipal solid waste were used as feedstock for gasification, ethanol production was rather low, owing to cyanide toxicity; meanwhile, when CO-rich flue gases from the steel industry were used, the project seemed to have successfully developed. Worth 47 ktons per year, this project was the first to produce ethanol by gas fermentation to be built in Europe. Consequently, pretreatment systems that are suitable for raw syngas fermentation need to be urgently developed (Liew et al. 2016).

4 Process Coupling and Perspectives

First, impurities such as NO_x in syngas need to be removed for the use in syngas fermentation. Conventional syngas upgrading includes cyclones (for particulate removal), water quench scrubbers for removal of ammonia and trace impurities, and mixed oxide sorbents for H_2S removal (Torres et al. 2007; Woolcock and Brown

2013; Xu et al. 2011). Shen et al. (2016) recently reviewed syngas cleaning processes and proposed that biochar and bio-oil can be potentially used for gas cleaning in biomass pyrolysis/gasification. Other techniques, such as membrane separation, may also be used to purify syngas (Castro-Dominguez et al. 2017; Parsley et al. 2014). Castro-Dominguez et al. (2017) demonstrated the pilot-scale application of palladium-based membrane technology for the purification of H_2 from coal-derived syngas; the results indicated that the purity of the produced H_2 ranged from 99.87 to 98% and that H_2 production of 2.72 kg/day and recovery of 64% were achieved.

Second, metabolites in syngas fermentation always consist of a mixture; thus, coupling processes are necessary to use the mixed products. As potential substitutes for petroleum fuel, ethanol has attracted more attention for their higher energy density, less corrosiveness, and higher compatibility with gasoline (Xue et al. 2013). For high volatility under high temperature, ethanol can be easily recovered by gas stripping after coupling with syngas fermentation. Löser et al. (2005) showed that more than 30% of produced ethanol in the reactor could be removed. Xue et al. (2016) recently developed two-stage gas stripping and pervaporation integrated with acetone–butanol–ethanol (ABE) fermentation for butanol recovery. The results indicated that considerably more ABE (27.5 g/L acetone, 75.5 g/L butanol, 7.0 g/L ethanol) were produced in fed-batch fermentation.

Third, electrodialysis (ED) is a traditional technology and can be used to separate and concentrate organic acids (Moresi and Sappino 2000; Zhang et al. 2011). Redwood et al. (2012) proposed an integrated hydrogen refinery of food wastes in a synergistic combination of photofermentation, extractive fermentation, and hydrothermal hydrolysis. In this process, ED provided the key link in waste to energy for the selective separation of organic acids. Zhang et al. (2009) proposed the use of a mixture of water and ethanol to be used as a medium for enhancing the solubility of sebacic acid, which can also facilitate the recovery of medium long-chain organic acids, such as caproate and caprylate; this technique requires further study. Except for the bacterial metabolites of organic acid and alcohol, the components of MCF broth normally include inorganic salts, which decrease the real separation factors, such as current efficiency. Zhang et al. (2011) analyzed the ion competition between organic acids (e.g., formate, acetate, propionate, and butyrate) and inorganic salts (e.g., HPO_4^{2-} and Cl^-) and found that membrane selectivity depended on the size, charge, and functional groups of the organic ions. The concentrations of acetate, propionate, and butyrate are decreased more slowly because of the presence of inorganic ions. Current efficiency was even lower than 30%; thus, the development of the selective separation of membranes for specific metabolites is urgently needed. Coupling of syngas fermentation with ED deserves further research.

Fourth, for a longer carbon chain and a lower O/C ratio, the mixture of the produced medium-chain fatty acids could also be upgraded to biofuels by hydrogen reduction (Steinbusch et al. 2011; Zhang et al. 2013b). The produced metabolites in syngas fermentation, such as acetate and ethanol, can be suitable substrates for the production of medium-chain fatty acids (Grootscholten et al. 2014; Kucek et al. 2016). Kucek et al. (2016) achieved high *n*-caprylate productivity (0.33 g/(L•day)) by feeding a high substrate ratio of ethanol to acetate amounting to 15 gCOD/gCOD

and extracting the product from the bioreactor broth. Xu et al. (2015) extracted *n*-caproate from the bioreactor broth by a hollow fiber membrane and found that selective phase separation occurred because of the low maximum solubility of this acid, which allowed the separation of simple products into an oily liquid containing 90% *n*-caproic and *n*-caprylic acids. However, the bacterial toxicities of medium-chain carboxylic acids still need to be considered (Zhang et al. 2013b). Khor et al. (2017) recently converted medium-chain fatty acids to decane (0.41$/Kg) via Kolbe electrolysis; the low density and low solubility of decane render it a rather simple product to target in terms of process engineering because the liquid fuel market is extensive and well-entrenched (Khor et al. 2017).

Lastly, apart from the metabolites shown in Fig. 1, syngas can also be converted to biopolymers, such as polyhydroxyalkanoates (PHA) (Revelles et al. 2016). Lagoa-Costa et al. (2017) recently proposed a two-stage syngas utilization system by using *C. autoethanogenum* and a highly enriched PHA-accumulating biomass that could convert syngas to ethanol, 2,3-butanediol, and PHA; the maximum PHA content was 24%. Meanwhile, MFC is a fast-growing environmental biotechnology in which bio-convertible substrates are consumed with simultaneous electron generation (Logan and Regan 2006; Schroder et al. 2015). Syngas can also be converted to electricity in MCF. Hussain et al. (2012) demonstrated electricity generation in a thermophilic MFC operated on syngas (CO and H_2, 50:50 v/v) as the sole electron donor, with volumetric power output ranging from 30 to 35 mW/L and syngas conversion efficiency ranging from 87 to 98%. Foley et al. (2010) showed that MFC provides no significant environmental benefit relative to conventional anaerobic treatment; by contrast, a microbial electrolysis cell provides significant environmental benefits for biochemical production. Consequently, syngas utilization in a microbial electrolysis cell may also need to be evaluated in future research. Thus, syngas fermentation provides a promising platform for biochemical production but requires other related methods, including membrane separation and MFC, to promote its application worldwide.

5 Conclusion

In this chapter, the basic bioreactions of the Wood–Ljungdahl pathway and reverse β-oxidation reaction and thermodynamics are summarized in Sect. 2. The operating conditions—pH, temperature, CO and H_2 partial pressure, and impurities of tar and NO_x—and the reactor configuration are reviewed in Sect. 3. Lastly, syngas fermentation coupled with other technologies, such as syngas pretreatment and membrane technology, was necessary for its application, as summarized in Sect. 4. Similarly, other high-potential technologies such as PHA production and MFC are also reviewed in Sect. 4. Thus, syngas fermentation provides a promising platform for biochemical production, but to promote its application, coupled technologies are still necessary.

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