

Review

Impacts of Syngas Composition on Anaerobic Fermentation

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Abstract: Energy consumption places growing demands on modern lifestyles, which have direct impacts on the world's natural environment. To attain the levels of sustainability required to avoid further consequences of changes in the climate, alternatives for sustainable production not only of energy but also materials and chemicals must be pursued. In this respect, syngas fermentation has recently attracted much attention, particularly from industries responsible for high levels of greenhouse gas emissions. Syngas can be obtained by thermochemical conversion of biomass, animal waste, coal, municipal solid wastes and other carbonaceous materials, and its composition depends on biomass properties and gasification conditions. It is defined as a gaseous mixture of CO and H₂ but, depending on those parameters, it can also contain CO₂, CH₄ and secondary components, such as tar, oxygen and nitrogenous compounds. Even so, raw syngas can be used by anaerobic bacteria to produce biofuels (ethanol, butanol, etc.) and biochemicals (acetic acid, butyric acid, etc.). This review updates recent work on the influence of biomass properties and gasification parameters on syngas composition and details the influence of these secondary components and CO/H₂ molar ratio on microbial metabolism and product formation. Moreover, the main challenges, opportunities and current developments in syngas fermentation are highlighted in this review.

Keywords: syngas fermentation; biomass composition; biomass gasification; hybrid processes; thermo-biochemical conversion



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1. Introduction

The increasing global demand for natural resources tends to exceed our Earth's capacity for their regeneration. As a result, environmental deterioration such as ocean acidification, greenhouse gas accumulation in the atmosphere and groundwater depletion, is accelerating [1,2]. Faced with this scenario, alternative energy and materials sources are needed to meet growing demands as well as society's environmental and economic concerns. Thus, sustainable goals set by several countries promote the use of bioenergy, biofuels and biochemicals [2,3].

Syngas fermentation fulfills the requirements for their sustainable production, having recently attracted much attention. It can be implemented directly in industries where the high levels of exhaust gases are constantly released, such as cement or steel manufacturing, oil refining and the production of petrochemicals, contributing to the reduction in greenhouse gas emissions [3,4]. Moreover, syngas can be obtained by thermochemical conversion, e.g., pyrolysis and gasification, of biomass, animal waste, coal, municipal solid waste and other carbonaceous materials [4].

Syngas, also named synthesis gas, is mainly composed of CO (carbon monoxide), CO₂ (carbon dioxide) and H₂ (hydrogen gas) which can be converted to alcohols such as ethanol, butanol and hexanol and other chemicals such as acetic, butyric and hexanoic acids by acetogenic bacteria through the Wood–Ljungdahl pathway or its derivatives [4,5]. Syngas fermentation is one of three major approaches to the production of second generation

biofuels, along with the Fischer–Tropsch process and lignocellulosic fermentation [6]. The combination of gasification with syngas fermentation results in taking advantage of some of the relevant features of thermochemical and biochemical technologies; however, the fermentation of these gaseous substrates presents challenges that still must be overcome. Syngas fermentation depends on factors such as the microorganism, type of reactor, gas composition, medium components, operating parameters, gas–liquid mass transfer and fermentation strategies [5].

The present review provides an update of this work. Special attention is dedicated to syngas composition since its impact on a microorganism's metabolism affects product distribution, titer, yield, productivity and the feasibility of the processes involved. Additionally, syngas composition depends on how it is produced, i.e., the process, operating conditions and raw materials, among others.

2. Syngas

Syngas is a gaseous mixture of CO, H₂ and CO₂. It also contains CH₄ while secondary components such as H₂O, H₂S or NH₃ or tar are often present [5,7]. It can be used to produce biofuels (gasoline, diesel oil), energy (heat and/or electricity generation) and chemicals [7].

Although syngas can be obtained from fossil-based sources like coal, natural gas, petcoke or crude oil fractions, by steam methane reforming (SMR), autothermal reforming (ATR), partial oxidation (POX), dry methane reforming (DMR), bi-reforming (BR), tri-reforming (TR) and combined reforming (CR); gasification is presently very attractive given that syngas can also be produced from a wide range of carbonaceous organic feedstocks, including biomass, municipal solid wastes (MSW) and plastics [8,9].

2.1. Gasification

Gasification occurs at high temperatures and in the presence of a limited amount of a gasifying agent (steam, air, oxygen, carbon dioxide or their mixtures), producing syngas by cracking organic compounds [7,10]. The gasification of different carbon-containing feedstocks using different gasifying agents and a wide spectrum of catalysts allows it to achieve specific targeted syngas quality and yield, which makes this technology very flexible and versatile. Gasification temperature, operating pressure, gasifier configurations, residence time, superficial velocity and flow rate of the gasifying agent are other operational factors that influence the composition and yield of syngas, expanding the syngas composition possibilities. Moreover, it is considered a sustainable thermochemical conversion technology for the production of clean gaseous fuels since several renewable feedstocks such as biomass, solid wastes and/or by-products, such as glycerol, from biorefinery processes can be used [7,9].

2.1.1. Gasification of Biomass

Sludge, energy crops, crop residues, wood, algal biomass and tamarind shells, among others, can be converted into gaseous products by gasification [7,11,12]. Compared to fossil sources, biomass contains less nitrogen, sulfur and heavy metals, and has higher H/C ratios, resulting in lower pollutant emissions, higher reactivity and lower gasification temperatures [10,13].

The conversion of biomass to syngas usually occurs under oxygen-limited and fuel-rich conditions through four steps: drying, pyrolysis, combustion and reduction. Drying, the first step, involves the release of moisture from biomass, which further reacts to produce hydrogen gas (water gas shift reactions). The biomass then undergoes thermal decomposition in the pyrolysis zone. Combustion, which is an exothermic reaction, is the next step wherein the devolatilized products (produced during pyrolysis) react with an oxidizing agent, producing the heat essential for endothermic reactions. Reduction is the final gasification step, which reduces the hot combustion products (produced during oxidation) mainly to CO and H₂ [7,10].

Biomass properties (such as size, shape, density, chemical composition, energy and moisture content, reactivity, presence of ash, alkali and volatile compounds) as well as gasification conditions (such as type and design of the reactor, temperature, pressure, type and flow of oxidizing agents, biomass flow and type and amount of catalyst) have considerable influence on syngas composition and impurities [7,10]. Table 1 lists syngas composition and impurities from the gasification of a variety of feedstocks as a function of biomass feedstock, oxidizing agent, gasifier reactor type, equivalence ratio and temperature.

Table 1. Syngas composition and impurities from gasification of a variety of feedstocks as a function of biomass feedstock, oxidizing agent, gasifier reactor type, equivalence ratio and temperature.

Feedstock	Gasifying Agent	Equivalence Ratio (ER)	Gasifier	Temperature	Gas Composition					Reference	
					H ₂ %	N ₂ %	CO%	CH ₄ %	CO ₂ %		Others
Cypress sawdust Mixed pine bark-spruce	air	0.54	fluidized bed	700–850 °C	5.6	68.0	6.9	1.4	18.1	tar: 15.3 g/Nm ³	[14]
		0.22			5.4	53.9	21.4	4.6	14.7		
Wood chips	air O ₂ steam steam (dry)	0.30	fluidized bed	800 °C	13.2	41.1	18.0	4.1	11.5		[15]
		0.30			27.9	0.9	35.7	0.76	10.5		
		n/a			31.1	0.0	14.0	7.3	14.1		
		n/a			41.0	0.06	18.5	9.7	18.6		
Rice husk	air	-	bubbling fluidized bed	702 °C	4.4	57.1	21.3	4.3	11.3	[16]	
		-		737 °C	4.8	57.1	16.9	3.7	15.9		
Wood biomass pellet Refused paper and plastic fuel	steam and O ₂	0.28	fluidized bed	750 °C	40.0	5.0	20.0	5.0	30.0	tar: 5 g/Nm ³	[17]
		0.31			35.0	2.0	20.0	8.0	30.0	tar > 10 g/Nm ³	
Sugar cane bagasse	super critical water	n/a	autoclave reactor	750 °C	45.0	5.0	5.0	10.0	40.0	[18]	
Miscanthus	steam	n/a	fluidized bed	815 °C	41.7	-	25.6	9.3	23.4	[19]	
Miscanthus	steam and O ₂	0.24	fluidized bed	800 °C	22.8	4.6	31.4	9.5	31.7	BTX:42 g/m ³ ; phenolics: 4.6 g/m ³	[20]
Straw		0.35			32.0	4.2	12.7	5.8	45.3	BTX:23 g/m ³ ; phenolics: 1.2 g/m ³	
Wood		0.28			21.8	4.6	33.7	8.9	31.0	BTX:38 g/m ³ ; phenolics: 2.2 g/m ³	
Rubber wood	air	-	fixed bed downdraft	600 °C	17.2	51.9	19.6	1.4	9.9	[21]	
Olive Peach Pine	air	-	fixed bed downdraft	1190 °C 1170 °C 1140 °C	13.2 15.0 12.0	54.9 51.7 59.4	17.4 17.7 16.0	0.8 1.2 0.2	12.4 13.5 11.4	[22]	
Beech wood	steam	n/a	bubbling fluidized bed	850 °C	33.4	-	28	8.8	23.8	13 g/Nm ³ tar, 0.05% C ₂ H ₆ , 1.6% C ₂ H ₄ , 0.03% C ₂ H ₂	[23]
Lignin-rich feedstock		n/a			35.5	-	19.8	11.4	24.4	21.2 g/Nm ³ , 0.67% C ₂ H ₆ , 3.3% C ₂ H ₄ , 0.11% C ₂ H ₂	
Sewage sludge *	Air and N ₂	0.10	fluidized bed	850 °C	33.30	-	26.16	11.46	20.90	C ₂ H ₄ , 7.99%; C ₂ H ₆ , 0.19%; tar; solid residues	[24]
		0.20			26.92	-	30.99	10.15	28.97	C ₂ H ₄ , 2.77% C ₂ H ₆ , 0.19%; tar; solid residues	

* Two different sewage sludge samples were used. The first was taken in January 2019 (ER = 0.2) and the second was taken in April 2019 (ER = 0.1); syngas composition was calculated based on N₂-free basis.

2.1.2. Gasification Parameters

Fixed and fluidized beds are the most used reactors for biomass gasification. Fixed bed gasifiers, usually used for slow conversion, operate at higher temperatures due to the non-uniform mixing of biomass, generating a high tar content of the producer gas. Fixed bed gasifiers involve an updraft or downdraft while fluidized bed configurations can include either circulating fluidized beds or bubbling beds. As a fluidized bed system works with the principle of a high velocity fluid flow, it is mainly used for fast conversion, operating at a homogeneous temperature. However, more of the particulate matter (char and ash) is generated by fluidized bed gasifiers as compared to fixed bed gasifiers [7,11].

The major advantage of the fluidized bed gasifiers is the high heat transfer rates it can handle, besides its temperature control and ease of operation. Thus, this reactor can be scaled up for industrial applications or large-scale production, whereas a fixed bed reactor is suitable for small scale production [7,11].

For each configuration and type of biomass, an optimum biomass flow rate is required for the gasification process in order to maximize energy efficiency. An overfeed of biomass reduces the conversion efficiency; however, biomass feeds below the optimum flow rate lead to lower gas yield. The flow rate of the gasifying agent is also important, especially when air or oxygen is used because it controls the degree of combustion and, hence, the reactor temperature. An increase in temperature promotes an increase in gas yield; however, it can also increase soot formation, especially in fixed bed gasifiers. Soot is an aerosol product formed by incomplete combustion, which reduces the energy efficiency and can interrupt continuous operation by causing blockages of pipes, hot-gas filters and heat exchangers, as well as by poisoning catalysts [7,13].

According to Murugan and Seklar [12], the performance of a gasifier depends on the moisture content of the feedstock and its equivalence ratio, which is the ratio of actual air supplied to the stoichiometric air required for complete combustion of the biomass. The authors studied the thermochemical gasification of tamarind shells (*Tamarindus Indica*) by numerical simulation and experimental observations, investigating the equivalence ratio and the feedstock moisture from 0.20 to 0.40 and from 10% to 18%, respectively. The results revealed that the calorific value of the producer gas reached a maximum (5.76 MJ/Nm³) when the equivalence ratio was 0.30. Under these conditions, the producer gas was composed of 22.49% CO, 14.48% H₂, 2.8% CH₄, and 15.88% CO₂, the average producer gas yield was 1.95 Nm³/kg, and the CO₂/CO ratio, cold gas efficiency and gas conversion efficiency were at a maximum of 0.69, 70.57% and 78.23%, respectively.

2.1.3. Biomass Composition

Lignocellulosic biomass, the most abundant source of renewable carbon on Earth, is composed of cellulose, lignocellulose and lignin, and a higher ratio of cellulose and hemicellulose to lignin generates a higher syngas yield [7]. Patel et al. [25] studied the gasification of lignite and waste wood mixtures in a pilot-scale fixed bed gasifier using air as the gasifying agent. Waste wood content was varied from 0 to 30% and the results indicate an increase in H₂ and CO₂ compositions in producer gas when the waste wood percentage increased, while CO and CH₄ compositions were not affected. The energy and exergy efficiencies, higher heating value and specific gas yield also increased when the wood content varied from 0 to 30%, increasing from 29.93% to 33.42%, 65.78% to 71.65%, 4.47 MJ/Nm³ to 4.75 MJ/Nm³ and 2.57 Nm³/Kg to 2.81 Nm³/Kg, respectively.

Liakakou et al. [23] compared the performance of lignin and beech wood in a system combining the biomass gasification (bubbling fluidized bed reactor) and syngas fermentation. Comparing the syngas obtained, H₂ production was significantly higher when lignin was used while CO was meaningfully higher in beech wood gasification. As a result, H₂/CO ratios of syngas were 0.80 and 1.27 for beech wood and lignin, respectively. Carbon dioxide and CH₄ productions were similar in both beech wood and lignin gasification.

Syngas composition is affected by biomass composition; however, the moisture content is also a relevant parameter. According to Khushboo et al. [7], energy efficiency increases

and syngas quality improves by reducing moisture content. Murugan and Sekhar [12] studied the influence of feedstock moisture on the producer gas composition. When the moisture of the tamarind shell varied between 10 and 18%, the compositions of CO, H₂ and CO₂ were 21.3–23.4%, 13.6–13.80% and 13.4–14.5% respectively. The cold gas and gas conversion efficiencies were observed at a maximum of 10% moisture, even though their variations in the range of operating conditions that were tested were not significant.

Besides moisture content, catalysts can also play an important role in biomass gasification. Pio et al. [26] studied the influence of three different low-cost catalysts on biomass-derived producer gas in a pilot-scale bubbling fluidized bed. In the absence of a catalyst, the producer gas was composed of 7.7–16.9% CO, 3.2–8.3% H₂, 0.5–3.4% CH₄ and 9.5–14.6% CO₂, with 2.4–4.3 MJ/Nm³ lower heating value, specific dry gas production between 1.0 and 1.8 Nm³ dry gas/kg biomass (dry basis), cold gas efficiency between 13.7 and 30.5% and carbon conversion efficiency between 30.7 and 50.9%. In the presence of the catalysts, the producer gas composition was 14.2–37.6% CO, 9.5–14.7% H₂, 2.6–3.5% CH₄ and 3.6–14.8% CO₂, with 3.9–6.3 MJ/Nm³ lower heating value, specific dry gas production between 1.4 and 2.0 Nm³ dry gas/kg biomass (dry basis), cold gas efficiency between 38.1 and 66.3% and carbon conversion efficiency between 56.8 and 86.6%.

Sethuraman et al. [27] investigated the effects of the nitrogen content of the biomass feedstock on the producer gas composition. In this study, tests were carried out in a pilot-scale fluidized bed gasifier using biomass feedstocks with different nitrogen contents varying from 0.14 to 1.75 wt%, and the results showed that there is a direct and proportional relationship between the nitrogen content of the biomass and ammonia in the producer gas.

Habibollahzade et al. [28] simulated biomass gasification using a range of feedstocks and different gasifying agents such as air, O₂, O₂-enriched air, steam, CO₂ or their mixtures. Higher carbon percentages in the biomass feedstock resulted in higher cold gas efficiencies for O₂-based and mixed agents, while the cold gas efficiency was not affected by carbon percentage at higher H/C ratios for a steam agent. On the other hand, when CO₂ was used as a gasifying agent, the cold gas efficiency was higher at lower carbon percentages and H/C ratios of the biomass feedstock. Regarding the exergy efficiency of biomass gasification, this increased when the carbon percentage in the biomass feedstock increased using O₂-based agents, but when steam and CO₂ were used as gasifying agents, the results were reversed. The authors concluded that, for biomass gasification using O₂ as a gasifying agent, a feedstock with the chemical formula of CH_{1.2}O_{0.495} is preferred, while CH_{1.5}O_{0.71} and CH_{1.2}O_{0.5} are suitable feedstocks for biomass gasification using steam and CO₂, respectively.

3. Syngas Fermentation

Autotrophic fermentation offers a more sustainable carbon-neutral alternative for the production of chemicals and fuels. Autotrophic microorganisms can fix CO₂ and/or CO and convert them to products by fermentation. To reduce CO₂, these microorganisms require ATP and reducing power (NADH), which can be provided through light, so they are named photoautotrophs, or through external inorganic electron donors, called chemolithoautotrophs [29].

3.1. Microorganisms

Photoautotrophic microorganisms are either oxygenic or anoxygenic. Their main difference is the ability to generate reducing power and a proton gradient through the water-splitting oxygen photosystems, which are present only in the oxygenic group. *Synechocystis* spp. and *Synechococcus* spp. are examples of oxygenic photoautotrophs. The anoxygenic group requires an inorganic electron donor such as H₂ or H₂S to generate the reducing power. *Rhodobacter sphaeroides* is an example of an anoxygenic microorganism [30].

Chemolithoautotrophic microorganisms absorb energy and generate reducing power by oxidizing inorganic compounds such as H₂, H₂S and ammonia (NH₃), being named hydrogen-oxidizing, sulfur-oxidizing and nitrogen-oxidizing microorganisms, respectively.

The electron donors are oxidized in the cell and the electrons are channeled into respiratory chains, producing energy. The electron acceptor of this metabolism can be the oxygen in aerobic microorganisms, or a variety of organic and inorganic molecules such as carbon dioxide, fumarate and nitrate in anaerobic microorganisms [31].

The autotrophic metabolism that is best characterized in the literature is the Calvin–Benson–Bassham cycle, an aerobic metabolism with oxygen as the electron acceptor. This CO₂-fixation pathway is present in photoautotrophs and in aerobic chemolithoautotrophs such as *Cupriavidus necator*. The Calvin cycle is ATP-inefficient, and the kinetic rate of several enzymes is low [32].

Among the autotrophic microorganisms, a promising group of the chemolithoautotrophs consists of the acetogens. An acetogen can be defined as an anaerobic bacterium which is able to fix CO₂ and/or CO through the Wood–Ljungdahl pathway, producing biofuels (e.g., ethanol, butanol and hexanol) and biocommodities (e.g., acetate, lactate, butyrate, hexanoate, 2,3-butanediol and acetone). The most efficient CO₂-fixation pathway is the Wood–Ljungdahl, being capable of saving ATP, even when operating close to the border of thermodynamic feasibility. It has become the most promising route for CO₂ and CO utilization in a biological approach. There are more than 60 strains of acetogens reported in the literature. Most of them can grow using CO₂ and H₂, and a few of them use only CO or both substrates [31].

Clostridium carboxidivorans [33,34], *Clostridium aceticum* [35], *Acetobacterium woodii* [36], *Blautia producta*, *Clostridium autoethanogenum* [37], *Clostridium difficile*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Eubacterium limosum*, *Moorella thermoacetica*, *Clostridium scatologenes*, *Clostridium coskatii* [38] and *Butyribacterium methylotrophicum* are examples of acetogenic carboxydrotrophic microorganisms [39–41].

3.2. Fermentation Pathways

Carbon capture and storage/sequestration (CCS) technologies involve capturing CO₂, transporting it to a storage site and depositing it where it will not reach the atmosphere. This concept is complemented by the use of this CO₂ as a building block for biochemical syntheses representing a feasible alternative for a movement in the direction of a sustainable chemical industry and to reduce CO₂ emissions into the atmosphere [42].

There are six different known metabolisms which are naturally capable of assimilating CO₂: (1) Calvin–Benson–Bassham cycle (CBB); (2) Reductive Tricarboxylic Acid cycle (rTCA); (3) Wood–Ljungdahl pathway; (4) 3-hydroxypropionate 4-hydroxybutyrate cycle (3HP-4HB); (5) the Dicarboxylate 4-hydroxybutyrate cycle (DC-4HB); (6) 3-hydroxypropionate bicycle (3-HP). Wood–Ljungdahl is believed to be the oldest biochemical pathway, responsible for producing biomass and ATP in the ancient world. It is the most energetically efficient pathway for CO₂ fixation, which explains the large volume of recent studies on acetogenic microorganisms [31,42].

Wood–Ljungdahl Pathway

The Wood–Ljungdahl pathway is also known as the reductive pentose phosphate pathway. Basically, in this pathway, CO₂ is reduced to carbon monoxide and formic acid or directly into a formyl group. The formyl group is reduced to a methyl group and then combined with the carbon monoxide and coenzyme A to produce acetyl-CoA, an important intermediate in biomass and biomolecule production (Figure 1).

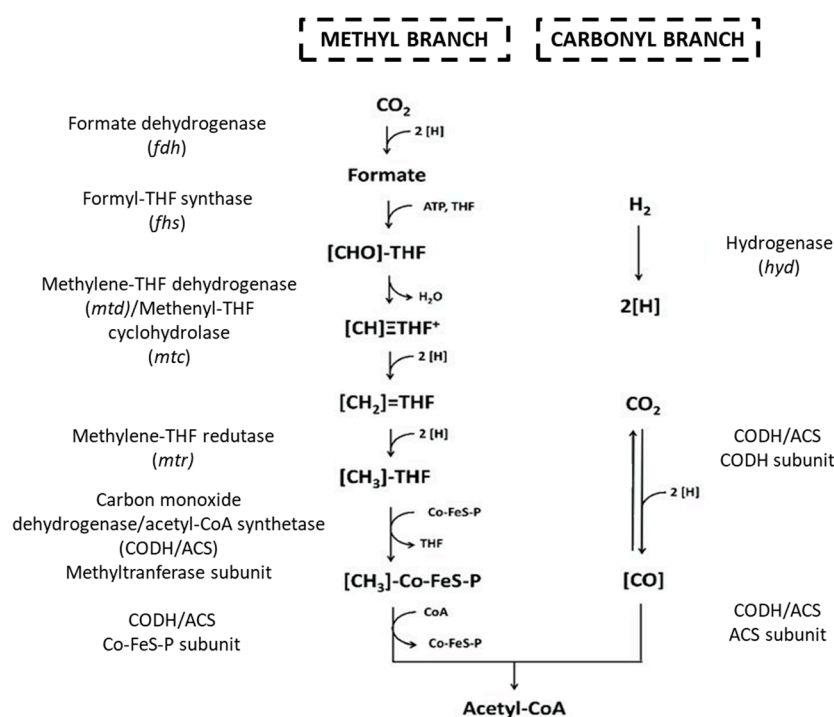


Figure 1. Wood–Ljungdahl pathway for CO_2 and CO conversion into acetyl-CoA by acetogenic bacteria based on [40,43].

The Wood–Ljungdahl pathway consists of methyl and carbonyl branches, as represented in Figure 1. In the methyl branch, one molecule of CO_2 is reduced to formate catalyzed by formate dehydrogenase (*fdh*). Subsequently, formate and tetrahydrofolate (THF) generate formyl-THF by the action of formyl-THF synthetase (*fhs*) in an ATP-consuming reaction. The reduction of formyl-THF to methyl-THF is catalyzed by methenyl-THF cyclohydrolase (*mtc*), methylene-THF dehydrogenase (*mtd*) and methylene-THF reductase (*mtr*). In the next step of the methyl branch, the methyl group is transferred to a corrinoid iron–sulfur protein. In the carbonyl branch, when CO is used as the only carbon source, one molecule of CO enters the carbonyl branch directly and another molecule of CO is oxidized to CO_2 by carbon monoxide dehydrogenase (CODH) with simultaneous formation of reduced ferredoxin. Finally, the enzyme complex carbon monoxide dehydrogenase/acetyl-CoA synthetase (CODH/ACS) forms the acetyl-CoA, coupling the methyl group, the carbonyl group and coenzyme A [5,31].

The acetyl-CoA formed by the Wood–Ljungdahl pathway can be used for biomass production or to undergo the action of phosphotransacetylase (*pta*) followed by acetate kinase (*ack*), generating 1 mol of ATP and 1 mol of acetate. Alternatively, acetyl-CoA can also be converted to acetaldehyde, then to ethanol; to butyryl-CoA and, subsequently, into butyrate and/or butanol; into hexanoyl-CoA and then hexanoate and/or hexanol, depending on the microorganisms used [44].

One important singularity of acetogenic microorganisms is the synergy between heterotrophic and autotrophic metabolisms, called mixotrophs. The CO_2 that results from sugar metabolization is reduced in a Wood–Ljungdahl pathway, using the reducing power from glycolysis to produce additional acetyl-CoA molecules. This results in the full fixation of carbon from sugar sources in a mixotrophic scenario [40]. Table 2 shows some results of syngas fermentation by acetogens reported in the literature.

Table 2. Results of syngas fermentation by acetogens reported in the literature.

Microorganism	Reactor	CO:H ₂ :CO ₂ :N ₂ :CH ₄	Impurities	Cell Growth (g Dry Weight Cell/L)	Products	Ref.
<i>Butyrivacterium methylothrophicum</i>	Serum bottles	100:0:0:0:0		<0.4	<0.6 g/L acetic acid; 0.4 g/L butyric acid; 0.07 g/L ethanol	[45]
		70:0:30:0:0		0.4	1.3 g/L acetic acid; 0.4 g/L butyric acid; 0.08 g/L ethanol	
		70:30:0:0:0		<0.4	<0.6 g/L acetic acid 0.6 g/L butyric acid; <0.02 g/L ethanol	
<i>Clostridium carboxidivorans</i>	CSTR	35:40:25:0:0		0.2	1 g/L acetic acid; 0.3 g/L butyric acid; 0.02 g/L ethanol	[44]
		20:10:20:50:0		0.42	2.7 g/L ethanol; 1.9 g/L butanol; 0.85 g/L hexanol	
<i>Clostridium carboxidivorans</i>	CSTR	50:15:35:0:0		<0.6	2 g/L ethanol; 1 g/L butanol; 0.5 g/L hexanol	[46]
<i>Clostridium carboxidivorans</i>	Serum bottles	50:15:35:0:0		<0.6	2.59 g/L acetic acid; 0.32 g/L butyric acid; 1.19 g/L ethanol; 0.18 g/L butanol	[47]
		80:0:20:0:0		0.74	1.86 g/L acetic acid; 2.52 g/L ethanol; 0.5 g/L butanol	
		60:0:40:0:0		1.8	3.35 g/L acetic acid; 1.8 g/L ethanol; 0.66 g/L butanol; 0.38 g/L hexanol	
<i>Clostridium carboxidivorans</i>	Serum bottles	25:44:10:10:11		0.47	2.3 g/L acetic acid; 1.9 g/L ethanol	[48]
		STBR	25:44:10:10:11		1.93	
<i>Clostridium carboxidivorans</i>	Serum bottles	16.5:5:15.5:56:4.5	no tar	0.35 (7 days)	2.2 g/L acetic acid; 0.12 g/L ethanol	[49]
		16.5:5:15.5:56:4.6	tar (C ₂ H ₂ , C ₂ H ₆ , C ₂ H ₄)	0.4 (11 days)	0.5 g/L acetic acid; 0.22 g/L ethanol	
<i>Clostridium ragsdalei</i>	CSTR	40:30:30:0:0	no impurities 0.37% NH ₃	0.52 0.41	significant reduction in hydrogenase activity with NH ₃ in the syngas	[50]
		32.5:32.5:16:19:0	no impurities	0.76	16.75 g/L acetic acid; 2.47 g/L ethanol	
<i>Clostridium ljungdahlii</i>	CSTR	32.5:32.5:16:19:0	150 ppm NH ₃ ; 54 ppb H ₂ S	0.71	10.27 g/L acetic acid; 3.29 g/L ethanol	[51]
		80:0:20:0:0	no impurities	0.4	0.96 g/L acetic acid; 1.17 g/L ethanol; 0.56 g/L butanol; 0.16 g/L	
<i>Clostridium carboxidivorans</i>	CSTR	80:0:20:0:0	0.1 g/L H ₂ S	0.76	0.8 g/L acetic acid; 3.2 g/L ethanol; 0.38 g/L hexanoic acid	[47]
		80:0:20:0:0	0.1 g/L NaNO ₃	0.6	0.38 g/L acetic acid; 1.1 g/L ethanol; 2.04 g/L butyric acid	
		80:0:20:0:0		0.6		

3.3. The Effect of Syngas Composition on Fermentation

As previously mentioned, syngas composition depends on process parameters, reactor design, gasifying agent, type of feedstock and its properties, besides the catalyst, when it is present [8,52]. Two aspects of its composition are particularly important for successfully integrating thermochemical and biochemical technologies, syngas impurities and H₂/CO ratios [10,23].

3.3.1. Syngas Impurities

Besides the major components (CO, H₂ and CO₂), biomass-derived syngas may contain additional constituents such as ethylene (C₂H₄), ethane (C₂H₆), acetylene (C₂H₂), tar, ash, char particles, oxygen (O₂), ammonia (NH₃), nitric oxide (NO), hydrogen sulfide (H₂S), sulfur dioxide (SO₂) and hydrogen cyanide (HCN) [53–55]. Tars can be composed of several high-molecular weight molecules that are gas chromatography undetectable, such as 7 carbon and higher ring compounds, heterocycles such as phenol, cresol and pyridine, light aromatic such as toluene, styrene and xylene, light poly-aromatic such as naphthalene, phenanthrene and anthracene, and heavy poly-aromatic such as fluoranthene, pyrene, chrysene, perylene and benzoperylene; however, usually these components are not evaluated individually [48]. The composition in terms of these impurities in syngas varies with feedstock type, gasifying agent and operating conditions [56], but the most frequently found compounds are tar, ammonia (NH₃), nitric oxide (NO) and hydrogen

cyanide (HCN) [53]. Some of these impurities can inhibit acetogenic bacterial activity, even at very low concentrations, by limiting cell growth, enzyme activities or by changing physiochemical conditions (pH, osmolarity, redox potential, etc.) [4]. Table 3 gathers some examples of investigations related to the effect of these impurities.

Despite the inhibiting or even toxic effects of these impurities on microbial growth and product formation, some authors report null or positive effects of some species. Rückel et al. [50] found that NH_3 and H_2S increased both growth and alcohol formation (ethanol, 1-butanol and 1-hexanol) during syngas fermentation by *Clostridium carboxidivorans*. On the other hand, Xu and Lewis [51] showed that NH_3 is rapidly converted to ammonium ion (NH_4^+) in the fermentation media and the accumulated NH_4^+ inhibits hydrogenase activity and cell growth of acetogenic bacteria. It seems that there is a microbial tolerance for these substances [10]. For example, *C. ragsdalei* was able to withstand 5% of oxygen [50].

Xu et al. [55] reported that entrained tar particulates larger than 0.025 mm, nitric oxide larger than 0.004 mol% and ammonia in general have an adverse effect on the fermentation process. Tars are mostly polynuclear hydrocarbons (such as pyrene and anthracene) that can clog engine valves, cause deposition on turbine blades or fouling of a turbine system [57]. Monir et al. [58] investigated bioethanol production through syngas fermentation in a tar free bioreactor using *Clostridium butyricum* and found that bacterial cell growth was 500 times higher when treated syngas was used instead of untreated syngas. Conventional scrubbing systems are generally the technology of choice for tar removal from the product syngas, but catalytically cracking reduces or eliminates this waste stream and also eliminates the cooling inefficiency of scrubbing, while enhancing the product gas quality and quantity [57]. Filtration can also be used successfully to remove tar, ash and other particulate matter from the biomass-derived producer gas [59].

Nitrogen oxide species, nitrate and nitrite, reduced biomass growth as well as alcohol concentrations for *Clostridium carboxidivorans* syngas fermentation [47]. Ahmed et al. [49] had already shown that nitric oxide, present in the producer gas at 150 ppm, is an inhibitor of the hydrogenase enzyme involved in H_2 consumption by this bacterium. The inhibitory effects of NO on syngas fermentation can be eliminated by improving the gasification efficiency or by scavenging it using agents such as sodium hydroxide, potassium permanganate or sodium hypochlorite [56].

Table 3. Influence of syngas impurities on microbial conversions and possible solutions.

Impurity (Conc.)	Process	Microorganism	Impurity Effect	Solution	Ref.
Benzene (327 mg/mL), toluene (117 mg/mL), ethylbenzene (131 mg/mL), p-xylene (92 mg/mL), and o-xylene and naphtha	Fermentation for ethanol/acetic acid production	<i>Clostridium carboxidivorans</i> P7	Cause of cell dormancy and product redistribution (more ethanol, less acetic acid)	Addition of filter in the gas cleanup	[49]
Acetone (2 g/L)	Isopropanol production from producer gas treated by wet scrubbing techniques using acetone.	<i>C. ragsdalei</i> (<i>Clostridium</i> strain P11), and <i>Clostridium carboxidivorans</i> P7	P11: Reduction of acetone to isopropanol; growth unaffected and ethanol concentrations increased by 55%; P7: no reduction of acetone; growth unaffected; 41% increase in ethanol and 79% decrease in acetic acid.	P11: opportunity for biological production of isopropanol from acetone with gaseous substrates	[60]
H ₂ S (1.0 g/L)	Bioconversion of CO-rich waste gases into short- and medium-chain alcohols	<i>C. carboxidivorans</i>	Positive effect on both growth and alcohol formation (ethanol, 1-butanol, and 1-hexanol).	-	[61]
NaNO ₃ (0.1 g/L from 2.2 g/L thioacetamide)	Bioconversion of CO-rich waste gases into short- and medium-chain alcohols	<i>C. carboxidivorans</i>	Reduce growth and 25% reduction of ethanol concentration	Reduction of NO _x components in syngas from the gasification and/or selectively removed	[61]
NaNO ₂ (0.5 and 0.1 g/L)	Bioconversion of CO-rich waste gases into short- and medium-chain alcohols	<i>C. carboxidivorans</i>	Strong toxic effect on the metabolism: no product formation	-	[61]
NH ₄ Cl (5.0 g/L)	Bioconversion of CO-rich waste gases into short- and medium-chain alcohols	<i>C. carboxidivorans</i>	Positive effect on both growth and alcohol formation (ethanol, 1-butanol, and 1-hexanol). Cell growth: more than 50% increase; 2x ethanol concentration	-	[61]
NO (150 ppm)	Fermentation for ethanol/acetic acid production	<i>Clostridium carboxidivorans</i> P7	Inhibitor of the hydrogenase enzyme involved in H ₂ consumption	Filter does not eliminate inhibition	[49]
NO (0–160 ppm)	Biomass-Generated Synthesis Gas	<i>Clostridium carboxidivorans</i> P7	NO < 40 ppm can be tolerated by cells in a syngas fermentation; NO > 40 ppm is a non-competitive inhibitor of hydrogenase activity (but it is reversible)	Use of syngas with NO < 40 ppm	[59]
NH ₃ (mole fraction of 0.37%)	Fermentation for biofuels production	<i>Clostridium ragsdalei</i> (<i>Clostridium</i> strain P11)	NH ₃ converts to ammonium ion (NH ₄ ⁺): inhibition of hydrogenase activity (at 650 mol/m ³ of [NH ₄ ⁺]: 50% of V ₀) and cell growth (cell density: 23% of the control at 227 mol/m ³ NH ₄ ⁺)	Remove NH ₃ impurity from raw syngas	[50]
O ₂ (400–26,000 ppm)	Syngas fermentation in a 100-L pilot scale fermentor	<i>Clostridium ragsdalei</i> (<i>Clostridium</i> strain P11)	Oxygen concentration in headspace (400 and 26,000 ppm): <i>Clostridium</i> strain P11 inoculum demonstrated growth and product formation.	-	[62]

3.3.2. H₂/CO Ratios

The thermochemical processes for syngas production can generate a wide range of H₂/CO ratios. For example, direct gasification of biomass using a fluidized bed gasifier provides a ratio of H₂ to CO between 0.25 and 0.53. Three gasification technologies (fixed bed updraft gasification with 30 kg/h solid feed, bubbling fluidized bed gasification with 0.3 kg/h solid feed and indirect gasification with 3 kg/h solid feed) resulted in quite different molar H₂/CO ratios ranging from 0.6 to 1.0 [23]. One of the advantages of syngas fermentation over chemical conversions to liquid fuels is the flexibility of feedstock and syngas composition because microorganisms do not require precise H₂/CO ratios [10].

Even with this flexibility identified for acetogens involved in syngas fermentation, a low ratio of H₂ to CO is preferred as most of the organisms grow better on CO than H₂ [23]. Lanzillo et al. [63] showed that partial pressure of CO affected the growth kinetics of the microorganism and the optimal P_{CO} within the studied range (0.5 to 2.5 atm) was 1.1 atm. Hurst and Lewis [64] demonstrated that increasing the partial pressure of CO from 0.35 to 2.0 atm, increased cell growth and the reduction of acetic acid into ethanol, and suggested that this is related to the potential importance of CO partial pressure and CO to CO₂ partial pressure ratio to electron and ATP production. However, increasing the concentration of H₂ in the gas phase can increase the contribution of CO to ethanol production because significant amounts of carbon from CO can be converted to cell material and ethanol if H₂ is utilized as an electron source. The depletion of H₂ in the fermentation medium would reduce the amount of carbon available for ethanol production because a fraction of CO would be utilized for generating the required reducing equivalents [59,65]. Valgepea et al. [66] proved that at the molecular level with quantitative proteome analysis, H₂ supply strongly impacts carbon distribution with a fourfold reduction in substrate loss and a proportional increase in flux to ethanol. Through metabolic modelling, they also showed that H₂ availability provided reducing power via H₂ oxidation and saved redox as cells reduced all the CO₂ to formate directly using H₂ in the Wood–Ljungdahl pathway.

Both CO and H₂ were utilized by *Clostridium ragsdalei*, also known as *Clostridium* strain P11, for growth, acetic acid and ethanol production, being 4.3 moles of CO and 0.45 moles of H₂ [65]. Ramachandriya et al. [60] reported that *Clostridium* strain P11 showed different CO₂ and H₂ consumption profiles compared to *Clostridium carboxidivorans* P7, or P7, although their CO profiles were similar. Hydrogen consumption by P11 was approximately 300% higher than P7. The authors considered the hypothesis that the low H₂ consumption by P7 could be related to a higher conversion of acetic acid into ethanol by this strain. The reduction of acetic acid into ethanol could be supported by the H⁺ gradient that replenishes the reducing equivalent pool, NAD(P)⁺ to NAD(P)H [60].

Jack et al. [67] evaluated syngas fermentation by *Clostridium ljungdahlii* on varying H₂/CO ratios and detected that the formation of acetate was favored by higher concentrations of hydrogen in the headspace. They also verified maximum acetate concentration (35.21 mM) using an H₂/CO ratio of 2.0 and maximum ethanol (7.53 mM) and 2,3-butanediol (5.20 mM) concentrations under an H₂/CO ratio of 0.5 [67]. The same process conditions of indirect gasification of lignin and beech wood resulted in H₂/CO ratios of 0.80 and 1.27, respectively, but similar acetate and ethanol productivities were obtained by *Clostridium ljungdahlii* DSM 13528 (acetate and ethanol: 0.16 and 0.02 g/L/h for lignin syngas and 0.17 and 0.01 g/L/h for beech wood syngas) [23].

Diender et al. [68] reported the use of a synthetic co-culture of *Clostridium autoethanogenum* and *Clostridium kluyveri* to convert syngas to a mixture of C₄ and C₆ fatty acids and their respective alcohols. Hydrogen gas and CO were co-utilized, which resulted in similar end products as from CO alone. Cultures with a higher CO/H₂ ratio produced relatively more chain-elongated products compared to cultures containing relatively less CO and utilized more acetate per mole of gas consumed.

4. Challenges and Opportunities for Syngas Fermentation

There are many issues that must be addressed in order to integrate biomass gasification and syngas fermentation into a large-scale technology. The main challenges include the carbon conversion efficiency of both steps, effects of gaseous impurities and the effect of H₂–CO–CO₂ ratios on syngas fermentation.

The overall carbon conversion efficiency can be calculated based on the carbon present in the feedstock that can be converted into organic fermentation products. Increasing the gasifier temperature converts tars into CO, CO₂ and H₂, thereby increasing the overall carbon conversion efficiency since these are fermentable gaseous species. The carbon conversion efficiency can also be controlled by the equivalence ratio. Higher equivalence ratios result in higher operating temperatures, which usually increase the degree of carbon oxidation and, thus, increase the carbon conversion efficiency of biomass gasification. However, the operating costs also increase as temperature increases. Moreover, higher equivalence ratios also result in more CO₂, while the optimum equivalence ratio results in more CO and H₂, which is more suitable for syngas fermentation, increasing the carbon conversion efficiency of syngas fermentation [10].

During syngas fermentation, CO and H₂ are the electron donors required for several reduction reactions of the metabolic pathway. Carbon monoxide seems to be thermodynamically the preferred source of electrons at any given pH, ionic strength, gas partial pressure and electron pair carrier [69]. However, the generation of reducing power from CO is undesirable because, besides an inefficient use of CO as an electron source (that could potentially be carried out by hydrogenase enzymes using H₂), it also diverts the carbon flux of CO towards CO₂ and away from ethanol or other products. Consequently, the carbon conversion efficiency decreases [10].

A convenient strategy could involve an increase in H₂ concentration in the gas phase which could promote its use as an electron source, increasing the availability of CO to produce cell material, ethanol and other products. As previously mentioned, if the H₂ concentration in the gas phase decreases, CO would be utilized to generate the required reducing equivalents, decreasing the amount of carbon available for the formation of organic products [59,65]. On the other hand, higher concentrations of H₂ in the headspace can favor acetate production over ethanol [68] since it increases the electron availability. In a low electron availability system, reducing equivalents can be provided by converting acetate into ethanol [60].

In short, even if microorganisms do not require rigorous H₂/CO ratios, this is an important parameter in order to obtain the desired products and high carbon conversion efficiencies. The ideal H₂/CO ratio can be obtained by adjusting the biomass feedstock, gasifying agent, type of gasifier and gasification parameters. The influence of feedstock composition and gasifying agent on syngas composition can be clearly observed in Table 1. Steam itself or steam with O₂ as a gasifying agent seem to produce higher amounts of H₂ compared to air or O₂, which means higher H₂/CO ratios [15,17]. High-lignin biomass gasification tends to produce more H₂ [17,23] compared to biomass with a low lignin content [12,16]. Nevertheless, using air or O₂ as a gasifying agent, the H₂/CO ratio decreases significantly [15,21].

In view of this scenario, the co-gasification of different types of biomass and the use of gasifying agent mixtures should be taken into consideration. A larger number of gasification studies would be recommended since there are large varieties in the biomass found around the world. The choice of biomass should also take into consideration other issues such as availability, price, accessibility, seasonality and compositional variability, among others.

5. Current Developments

Although syngas fermentation is not yet operating at an industrial scale, companies, mainly located in the United States, have been developing syngas fermentation processes

for the commercial production of biofuels and valuable chemicals using anaerobic bacteria, such as Synata Bio, LanzaTech, INEOS BIO and JUPENG BIO [70,71].

Synata Bio incorporated the technology of Coskata, Inc. in 2016, which combines the conversion of lignocellulosic residues, waste, coal, industrial gases and natural gas into syngas with the conversion of this syngas into biofuels (ethanol and butanol) and chemicals (propanol and carboxylic acids) using *Clostridium ragsdalei*, *Clostridium carboxidivorans* and *Clostridium coskatii*. LanzaTech Inc. ferments the gas from hydrothermal vents, waste gas from steel mills and coal producers and woody biomass containing CO, CO₂, H₂, CH₄ and H₂S. The company produces ethanol, butanol and 2,3-butanediol using *Clostridium autoethanogenum*. INEOS Bio produces ethanol and power via the gasification of lignocellulosic and household waste using *Clostridium ljungdahlii* [72].

Regarding syngas fermentation, INEOS BIO S.A. holds three patents registered in the US (US 20130316420; US 20140080195; US 20100227377) while Coskata, Inc. registered two (US 20130337513; US 20130177957) and LanzaTech, Inc. only registered one patent on syngas fermentation in the US (US 20120052541). JUPENG BIO, Inc. filed a patent application in 2019, but it is still under consideration [70].

According to Espacenet Database, Coskata, Inc. is the biggest patent-holding company regarding syngas fermentation in the world, with most of them registered in the US and Canada. Synata Bio, LanzaTech, Inc. and INEOS BIO S.A. have also registered a significant number of patents that report syngas fermentation. However, JUPENG BIO has filed a notable number of patent applications in the last two years, indicating its effort in developing a commercial syngas fermentation process [71].

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