



Review

# Syngas Derived from Lignocellulosic Biomass Gasification as an Alternative Resource for Innovative Bioprocesses

Cosetta Ciliberti <sup>1</sup>, Antonino Biundo <sup>1,2</sup>, Roberto Albergo <sup>3</sup> , Gennaro Agrimi <sup>1,2</sup> ,  
Giacobbe Braccio <sup>3</sup>, Isabella de Bari <sup>3</sup> and Isabella Pisano <sup>1,2,\*</sup> 

<sup>1</sup> Department of Bioscience, Biotechnology and Biopharmaceutics, University of Bari, Via Edoardo Orabona, 4, 70125 Bari, Italy; cosettacilib96@gmail.com (C.C.); antonino.biundo@gmail.com (A.B.); gennaro.agrimi@uniba.it (G.A.)

<sup>2</sup> Interuniversity Consortium for Biotechnology (CIB), 34100 Trieste, Italy

<sup>3</sup> Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Division of Bioenergy, Biorefinery and Green Chemistry, C.R. Trisaia S.S. 106 Jonica, 75026 Rotondella (MT), Italy; roberto.albergo@enea.it (R.A.); giacobbe.braccio@enea.it (G.B.); isabella.debari@enea.it (I.d.B.)

\* Correspondence: isabella.pisano@uniba.it

Received: 3 November 2020; Accepted: 24 November 2020; Published: 28 November 2020



**Abstract:** A hybrid system based on lignocellulosic biomass gasification and syngas fermentation represents a second-generation biorefinery approach that is currently in the development phase. Lignocellulosic biomass can be gasified to produce syngas, which is a gas mixture consisting mainly of H<sub>2</sub>, CO, and CO<sub>2</sub>. The major challenge of biomass gasification is the syngas's final quality. Consequently, the development of effective syngas clean-up technologies has gained increased interest in recent years. Furthermore, the bioconversion of syngas components has been intensively studied using acetogenic bacteria and their Wood–Ljungdahl pathway to produce, among others, acetate, ethanol, butyrate, butanol, caproate, hexanol, 2,3-butanediol, and lactate. Nowadays, syngas fermentation appears to be a promising alternative for producing commodity chemicals in comparison to fossil-based processes. Research studies on syngas fermentation have been focused on process design and optimization, investigating the medium composition, operating parameters, and bioreactor design. Moreover, metabolic engineering efforts have been made to develop genetically modified strains with improved production. In 2018, for the first time, a syngas fermentation pilot plant from biomass gasification was built by LanzaTech Inc. in cooperation with Aemetis, Inc. Future research will focus on coupling syngas fermentation with additional bioprocesses and/or on identifying new non-acetogenic microorganisms to produce high-value chemicals beyond acetate and ethanol.

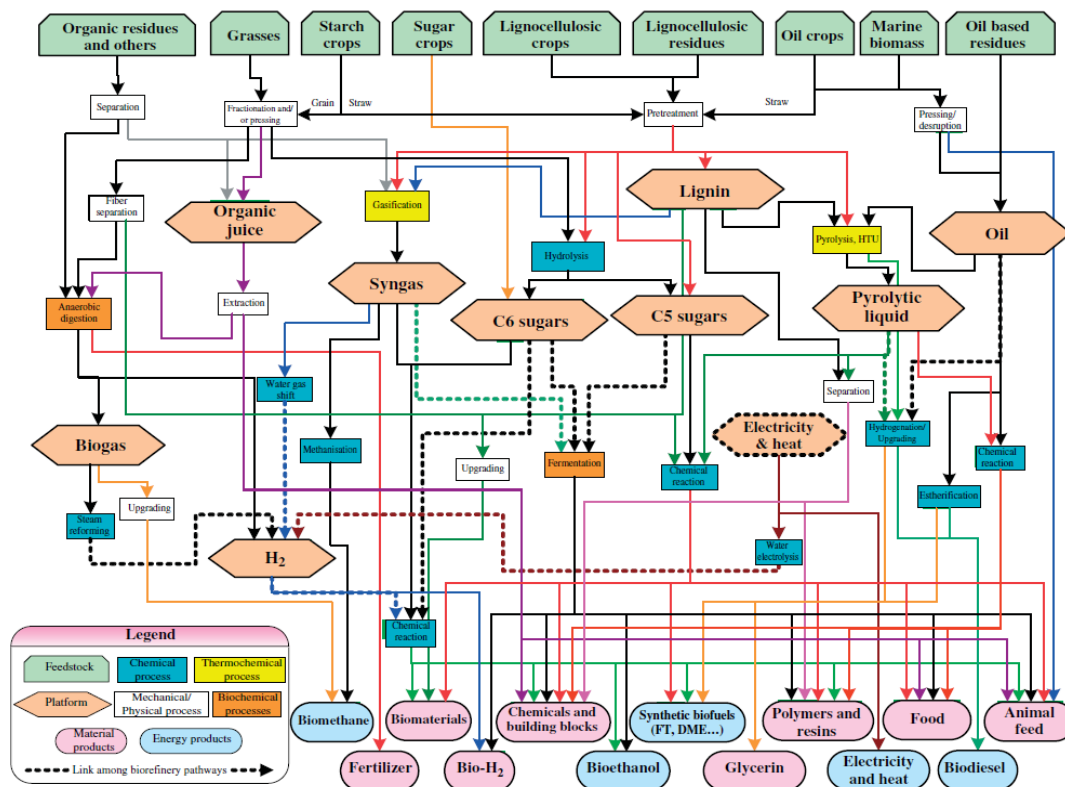
**Keywords:** gasification; syngas fermentation; lignocellulosic biomass; biorefinery; Wood–Ljungdahl pathway

## 1. Introduction

Nowadays, the current economic system is based on fossil resources, which include crude oil, coal, and natural gas. Crude oil is the most globally used resource. In order to produce energy, heat, and fuels (solid, liquid, gaseous), the global demand for oil is about 84 million barrels/day, which is estimated to reach approximately 116 million barrels/day in 2030 [1]. Moreover, commodity chemicals are mainly produced via oil refining [1]. However, fossil resources use is no longer economically and environmentally sustainable. From 1970 to 2017, the annual global extraction of materials (i.e., fossil resource, metals, non-metal minerals, and biomass) tripled. The latter continues

to grow due to the population's exponential growth, the increasing consumption of energy per capita, the economic and technological development, and the establishment of a new modern way of life [2]. Current scenarios show the increased fossil resources demand and the relative market price, and consequently, a negative impact on the environment due to the high amount of greenhouses gases (GHGs) emitted, i.e., carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), and water steam (H<sub>2</sub>O), which accumulate into the atmosphere, promoting global warming with environmental and economic consequences via melting glaciers, rising sea levels, ocean acidification, desertification, acid rains, and extreme local climate events [3].

The industry, energy, building, and mobility sectors account for the majority of GHG emissions in the European Union (EU). In December 2019, the European Commission launched the New Green Deal, which is a roadmap for making the European Union economy modern, resource-efficient, and competitive [4]. The Green Deal is an integral part of the European Commission's strategy to implement the United Nations 2030 Agenda and the Sustainable Development Goals (SDGs) [5]. According to the European Green Deal, the EU will be climate neutral by 2050 and economic growth will be decoupled from fossil resources use. It is a new growth strategy that aims to guide the transition of all sectors into a circular economy, the phasing out of fossil fuels through the introduction of alternative renewable resources for energy and chemical production, and reducing the environmental footprint of human activities [6]. While energy and heat can be produced using different types of resources, such as solar, wind, hydropower, and geothermal energy, biomass is the only resource that can be used to produce chemicals and materials in addition to energy. Biomass is the only carbon-rich resource on Earth, in addition to fossil resources [7]. European Directive 2009/23/EC has defined biomass as "the biodegradable fraction of products, waste and residues from biological origin from agriculture (including plant and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste" [8]. International Energy Agency (IEA) Bioenergy Task 42 has defined biorefining as "the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)." Different biomass conversion processes are integrated with each other in this system to achieve a wide spectrum of products without waste generation [1]. Biorefining is one of the main drivers for the establishment of a bio-based economy. In the first global bioeconomy summit in Berlin in November 2015, the Food and Agriculture Organization (FAO) of the United Nations defined a bioeconomy as the "knowledge-based production and utilization of biological resources, biological processes and principles to sustainably provide goods and services across all economic sectors" [9]. Biorefining has the following goals: (i) increase industry competitiveness and prosperity, (ii) decouple the economy from fossil resources, (iii) reduce GHG emissions, and (iv) improve local and rural development. Through efficient strategies, biorefining should be designed to achieve economic, social, and environmental sustainability [10]. Based on IEA Bioenergy Task 42, biorefineries can be classified according to four features: feedstocks, conversion processes, products, and platforms [11]. Therefore, biorefining can be considered as a system in which products are obtained from feedstocks, through platforms and conversion processes. Interconnections between the four elements co-create integrated systems of two or more biorefineries (Figure 1).



**Figure 1.** Overview of the biorefinery classification system. The network is organized into three levels: the upper level shows the specific feedstock which feeds each biorefinery, the intermediate level shows the platforms, and the lower level shows the products. Conversion processes can be applied to different feedstocks or platforms and they are linked through arrows ([11]; permission obtained from Wiley Online Library). HTU, Hydrothermal upgrading process, FT, Fischer Tropsch biofuels, DME, Dimethyl ether.

Moreover, based on technology implementation, biorefineries can be classified as first-, second-, and third-generation biorefineries.

First-generation biorefineries, which are based on edible plant biomass as feedstock, have shown the possibility of large-scale production, distribution, and use of biofuels produced from biomass [12]. The agri-food competition of first-generation biorefineries raises social and ethical issues, which motivate the development of second-generation biorefineries, a more flexible platform based on non-edible lignocellulosic biomass with a wider spectrum of products [10,12,13]. Third-generation biorefineries, whose feedstock is macro- and microalgae, are advantageous due to there being no need for fertile soil and the ability of algae to grow in the presence of a high amount of carbon dioxide in wastewater from industrial processes or saltwater [14]. Nevertheless, technology scale-up and product commercialization are the major challenges that compromise their techno-economic feasibility [15].

Therefore, a second-generation biorefinery is the most promising system for a bio-based economy. Indeed, lignocellulosic biomass is an ideal feedstock because it is widespread, economical, and easy to use. In addition, the use of this biomass is more efficient in a second-generation biorefinery due to the simultaneous production of biofuels, chemicals, electricity, and heat [16].

Lignocellulosic biomass can be obtained from either dedicated crops, such as woody and perennial herbaceous crops, or agricultural and industrial residues [1]. Lignocellulosic feedstocks have crucial advantages over other biomass feedstocks. They are the non-edible portion of the plant, and therefore, they do not interfere with food supplies. Moreover, high amounts of this biomass can be produced quickly and cheaply. Lignocellulosic biomass is mainly composed of three polymers: cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%) [17]. In addition to these components, the biomass also contains proteins, minerals, extractable compounds, and ash at lower concentrations [18]. Based on

the type of lignocellulosic biomass, the three polymers are organized into non-uniform complex 3D structures that are characterized by their robustness, recalcitrance, and resistance to degradation.

Lignocellulosic gasification is a valid thermochemical approach for the conversion of organic solid matter into a gaseous mixture that is constituted of  $H_2$ ,  $CO$ ,  $CO_2$ , and  $CH_4$ , named synthetic gas or syngas. Although about 55% of syngas is still produced from coal, biomass utilization, especially lignocellulose, is constantly growing [19]. Indeed, gasification could be potentially applied to all different kinds of lignocellulosic biomass, unlike other conversion technologies [20,21]. Moreover, in the last few decades, a wide range of applications of syngas have been intensively studied. Syngas can be directly used as a combustible substance in power plants for heat and power production (steam cycle, co-combustion, combustion in gas turbines or internal combustion engines, high-temperature fuel cells), which represents the most common use of biomass-derived syngas. However, syngas also represents a platform that can be employed in a broad range of chemical and microbial processes, leading to gaseous and liquid fuels, as well as to chemicals [22]. Chemical process research has mainly focused on transportation fuel production from syngas, such as Fischer–Tropsch liquid fuels, hydrogen, methanol, dimethyl ether (DME), mixed alcohols, and synthetic natural gas (SNG) [23]. Instead, the biochemical conversion route consists of syngas fermentation in which obligate anaerobic microorganisms convert syngas into organic acids, alcohols, and other chemicals (Figure 2). The most commonly used microorganisms are acetogens, which use the Wood–Ljungdahl metabolic pathway. Syngas fermentation is defined as an indirect fermentation process because biomass is not fed directly into the fermenter, but it is previously converted into syngas through gasification [24]. Biological catalysts, especially acetogenic microorganisms, that are used in syngas fermentation enable high reaction selectivity and high conversion efficiency, with increased product formation [20]. The aim of this review was to investigate the hybrid system based on biomass gasification, with a specific focus on alternative feedstocks and on syngas fermentation, providing detailed information on acetogenic microorganisms and their metabolism, process optimization, and bioreactors design. Recent advances in the field of the metabolic engineering of acetogens for the production of wide-ranging valuable compounds are addressed. Currently, only two companies, LanzaTech Inc. and Aemetis, Inc. in cooperation, have scaled up this technology, thereby demonstrating the techno-economic feasibility of this new concept of a biorefinery at a large scale. A brief description of their industrial plants is shown. Finally, this review describes the challenges and highlights the research gaps for future work in the field of syngas biorefining.

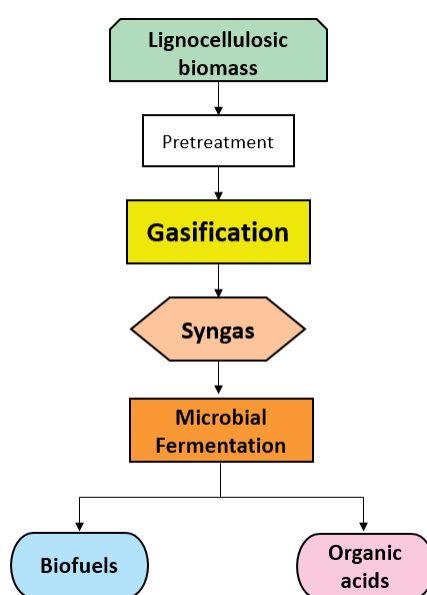
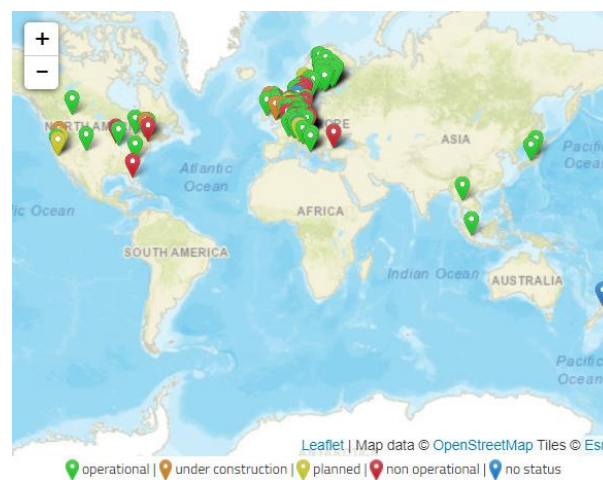


Figure 2. Syngas biorefinery concept.

## 2. Biomass Gasification

The gasification of carbonaceous feedstocks to syngas takes place inside a reactor, defined as a gasifier, at high temperatures (800–1500 °C). The feedstock is subjected to partial oxidation due to a lower concentration of oxygen than the stoichiometric requirement. Oxygen is supplied by a gasifying agent or carrier, such as air, pure oxygen, water steam, or their mixture. Although carbon dioxide can also be used as a gasifying agent, its use is less frequent. Moreover, the use of supercritical water is an innovative technology, without the need for pretreatment, which achieves a high H<sub>2</sub> yield and reduces tar and char production [25–27]. Compared with conventional methods, gasification is a more efficient process than combustion, which is the most common thermochemical route [28], and it can convert the entire carbon content in the biomass feedstock into gaseous compounds, unlike the biological or chemical hydrolysis that is adopted in biochemical processes [29]. According to the IEA Bioenergy Task 33, there are 114 working biomass gasification projects worldwide, 15 plants idle or on hold, and 13 are under construction or in planning (Figure 3) [30].



**Figure 3.** Geographical distribution of biomass gasification projects [30].

One main limitation of gasification technology is represented by the formation of tar. Tars are classified into primary, secondary, and tertiary tars. Primary tars consist of both oxygenated compounds (alcohols, carboxylic acids, ketones, aldehydes, etc.) and substituted phenols (cresol, xyleneol, etc.). Secondary tars are alkylated aromatics, such as toluene, ethylbenzene, xylenes, styrene, and hetero-aromatics, such as pyridine, furan, dioxin, and thiophene. Finally, tertiary tars consist of aromatics and polycyclic aromatic hydrocarbons (PAH), such as benzene, naphthalene, phenanthrene, pyrene, and benzopyrene. While primary tars are produced directly from the pyrolysis of cellulose, hemicellulose, and lignin, secondary and tertiary tars are the result of several complex reactions that have not been fully clarified yet [22]. At the end of the entire process, two main product mixtures are present: a solid mixture and a gaseous mixture. The solid mixture contains the unreacted organic fraction and inert materials, such as tars and ashes. The gaseous mixture contains syngas and a small amount of impurities, such as light hydrocarbons (ethane, ethylene, acetylene), hydrogen sulfide (H<sub>2</sub>S), sulfur dioxide (SO<sub>2</sub>), hydrogen chloride (HCl), nitrogen oxides (NO<sub>x</sub>), nitrogen (N<sub>2</sub>), and ammonia (NH<sub>3</sub>) [31]. The syngas's final composition and characteristics are related to the type of biomass, gasifying agent, gasifier type, and reactor's operational conditions, such as temperature, pressure, equivalence ratio (ER), residence time, and catalyst used [26,32–36]. For these reasons, in the last few decades, gasification has been intensively studied to investigate the effects of these factors, and thus, to identify the optimal conditions for the process. Regarding feedstock type, wood is the most commonly used feedstock in the gasification process. A representative component profile for syngas produced from several woody biomass types is shown in Table 1. In addition to woody biomass, other kinds of biomass have also been studied as gasification feedstocks. Agro-industrial

residues and perennial herbaceous crops (Table 2) represent promising feedstocks that can be used in a thermochemical conversion process to obtain both energy and chemicals. The use of agricultural and industrial wastes, as well as herbaceous crops, instead of woody feedstocks, extends the seasonal availability of biomass. A syngas's composition is highly dependent on the used feedstocks, as well as the gasification technology applied (Tables 1 and 2). It is worth pointing out that nitrogen ( $N_2$ ) can represent a main syngas component when air is used as a gasifying agent, in addition to  $H_2$ , CO,  $CO_2$ , and  $CH_4$ . Therefore, air gasification results in  $N_2$ -diluted syngas with low  $H_2$  and CO concentrations. Instead, when gasification is carried out with steam or oxygen, the syngas shows higher  $H_2$  and CO concentrations. The latter condition is the most suitable for syngas fermentation due to the fact that microorganisms use  $H_2$  and CO as primary substrates, in addition to  $CO_2$ . Although data on the negative effect of  $CH_4$  have not been reported, this component is not used by microorganisms during syngas fermentation. Therefore, the  $CH_4$  concentration in the syngas should be as low as possible. All the aforementioned impurities produced during the process can reduce the fermentability of syngas due to their negative effects on microorganisms. Therefore, one of the major challenges in biomass gasification is producing syngas with a low or absent impurities content. Biomass gasification needs further investigation studies to achieve an ideal syngas composition, thereby making the syngas fermentation process as efficient as possible [37].

**Table 1.** Syngas compositions that are obtained from the gasification of several woody biomass and their process characteristics.

Feedstock	Syngas Composition (% <i>v/v</i> )				Gasifier Type	Gasification Conditions	Reference
	H <sub>2</sub>	CO	CO <sub>2</sub>	CH <sub>4</sub>			
Mesquite wood	1.6–3.0	13.0–21.0	11.0–25.0	1.0–1.5	Fixed bed gasifier	GA: air; T: 782 °C; ER: 2.70	[38]
Juniper wood	2.5–3.5	21.0–25.0	9.0–12.0	1.5–1.8	Fixed bed gasifier	GA: air; T: 713 °C; ER: 2.70	[38]
Pine wood	30.5	52.8	14.7	2.0	Downdraft fixed bed gasifier	GA: steam; T: 900 °C; ER: N.A.	[39]
Oak wood	18.0	21.0	12.0	2.0	Downdraft fixed bed gasifier	GA: air; T: N.A.; ER: N.A.	[40]
Poplar wood	45.5	23.1	20.8	8.6	Rotary kiln reactor	GA: steam; T: 1500 °C; ER: N.A.	[41]
Eucalyptus wood	10.7	20.2	9.1	8.6	Downdraft fixed bed gasifier	GA: air; T: 865 °C; ER: 0.31	[42]
Coffee wood	12.4	14.0	10.4	6.5	Downdraft fixed bed gasifier	GA: air; T: 813 °C; ER: 0.32	[42]
Rubber wood	6.0–8.0	10.0–14.0	16.0–18.0	N.A.	Bubbling fluidized bed gasifier	GA: air; T: 750–900 °C; ER: 0.38	[43]
Oil palm wood	60.0–70.0	10.0–30.0	20.0–50.0	5.0–10.0	N.A.	GA: steam; T: 800 °C; ER: N.A.	[44]
Spruce wood	10.7	25.9	9.7	3.8	Fixed bed reactor	GA: air; T: 800 °C; ER: N.A.	[45]
Wood residue	42.5	23.0	18.1	11.5	Fluidized bed gasifier	GA: air; T: 823 °C; ER: 0.17	[46]
Vermont wood <sup>a</sup>	28.6	23.5	24.0	15.5	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Wood residue <sup>b</sup>	26.2–28.0	50.0–60.3	12.7–23.3	0.9–1.8	Entrained flow gasifier	GA: oxygen; T: 1200–1500 °C; ER: 0.44	[48]
SRF wood <sup>c</sup>	15.7–16.5	15.9–17.2	14.3–15.1	2.6–2.7	Downdraft fixed bed reactor	GA: air; T: 650–800 °C; ER: 0.25–0.26	[49]
Wood waste <sup>d</sup>	9.4–14.8	15.1–19.4	11.0–15.8	3.2–4.3	Bubbling fluidized bed gasifier	GA: air/air and steam mixture; T: 850 °C; ER: 0.20–0.29	[50]

GA: gasifying agent, T: temperature, ER: equivalence ratio, N.A.: data not available. <sup>a</sup> Vermont wood is a mixture of 25% red oak, 15% white pine, 15% maple, 15% ash, and 10% poplar, with the balance being cherry, birch, and cedar. <sup>b</sup> Wood residue is a mixture of 45% hardwood (birch) and 55% softwood (pine). <sup>c</sup> Solid recovered fuels (SRF) wood is composed of waste furniture and waste pallets from a waste collection site. <sup>d</sup> Wood waste that cannot be utilized to produce fuel for domestic heating because they come from potentially contaminated waste; it is made of sawdust from the wood packaging industry or it is obtained as a recycled product from furniture and from door and window frames.

**Table 2.** Syngas compositions obtained from the gasification of several agro-industrial residues and herbaceous crops and their process characteristics.

Feedstock	Syngas Composition (% <i>v/v</i> )				Gasifier Type	Gasification Conditions	Ref.
	H <sub>2</sub>	CO	CO <sub>2</sub>	CH <sub>4</sub>			
Corn straw	48.5	33.9	12.2	5.3	N.A.	GA: N.A.; T: 750–900 °C; ER: N.A.	[51]
Wheat straw	25.4	27.5	22.0	16.3	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Rice husk	5.0–8.0	16.0–21.0	15.0–16.0	46.0	Fluidized bed gasifier	GA: air; T: 700–800 °C; ER: 0.18–0.27	[52]
Coffee husk	6.6	13.8	12.1	14.8	Downdraft fixed bed gasifier	GA: air; T: 669 °C; ER: 0.12	[42]
Coconut coir	7.0–21.4	18.6–20.3	19.1–21.3	6.1–9.0	Entrained flow reactor	GA: air; T: 726–941 °C; ER: 0.21–0.30	[53]
Groundnut shells	13.8	13.0	13.5	5.7	Bubbling fluidized bed gasifier	GA: air; T: 714.4 °C; ER: 0.31	[54]
Almond shells	34.2–39.6	17.8–23.2	10.7–16.8	N.A.	Bubbling fluidized bed reactor	GA: N.A.; T: 820 °C; ER: N.A.	[55]
Hazelnut shells	11.1–14.7	8.6–20.7	9.5–16.3	1.4–2.5	Downdraft fixed bed gasifier	GA: air; T: 1000–1050 °C; ER: N.A.	[56]
Hay	8.8	19.7	14.4	3.0	Fixed bed reactor	GA: air; T: 800 °C; ER: N.A.	[45]
Corn stover	26.9	24.7	23.7	15.3	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Olive kernels	5.4–9.3	6.9–8.6	19.0–21.7	1.8–3.0	Circulating fluidized bed gasifier	GA: air; T: 800 °C; ER: 0.4–0.7	[57]
Vine pruning	17.1–18.4	21.3–21.7	11.3–13.0	2.1–2.6	Downdraft fixed bed reactor	GA: air; T: N.A.; ER: 0.26	[58]
Corncoobs	17.3	22.6	12.0	1.98	Downdraft fixed bed reactor	GA: air; T: N.A.; ER: 0.28	[59]
Citrus peels	60.0–65.0	15.0–25.0	15.0–23.0	<5.0	Fixed bed gasifier	GA: steam; T: 750 °C; ER: N.A.	[60]
<i>Posidonia oceanica</i>	11.8–24.9	4.1–12.7	14.1–20.0	2.0–3.0	Fluidized bed gasifier	GA: air; T: 750 °C; ER: 0.3	[61]
Empty fruit brunch	12.9–13.5	17.0–17.4	13.7–14.5	1.5–1.9	Downdraft fixed bed gasifier	GA: air; T: 650–825 °C; ER: N.A.	[62]
Sugarcane bagasse	7.4–8.0	8.0–12.9	15.9–18.7	1.4–2.5	Cyclone gasifier	GA: air; T: 600–950 °C; ER: 0.18–0.25	[63]
Sewage sludge	5.1–8.1	19.5–31.6	13.3–16.5	0.9–1.5	Fixed-bed gasifier	GA: air; T: 650–1100 °C; ER: 0.12–0.27	[64]
<i>Miscanthus X giganteus</i>	8.6	16.4	14.0	4.4	Bubbling fluidized bed reactor	GA: air; T: 800 °C; ER: 0.21	[65]
Switchgrass ( <i>Panicum virgatum</i> )	23.5	33.2	19.4	17.0	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Thistle ( <i>Cynara cardunculus</i> L.)	36.6	8.5	50.4	4.5	Circulating fluidized bed gasifier	GA: steam and oxygen; T: 750 °C; ER: 0.3	[66]
Wheatgrass ( <i>Elytrigia elongata</i> )	10.8	12.3	16.5	5.3	Bubbling fluidized bed gasifier	GA: oxygen-enriched air; T: 800 °C; ER: N.A.	[67]

GA: gasifying agent. T: temperature. ER: equivalence ratio. N.A.: not available data.



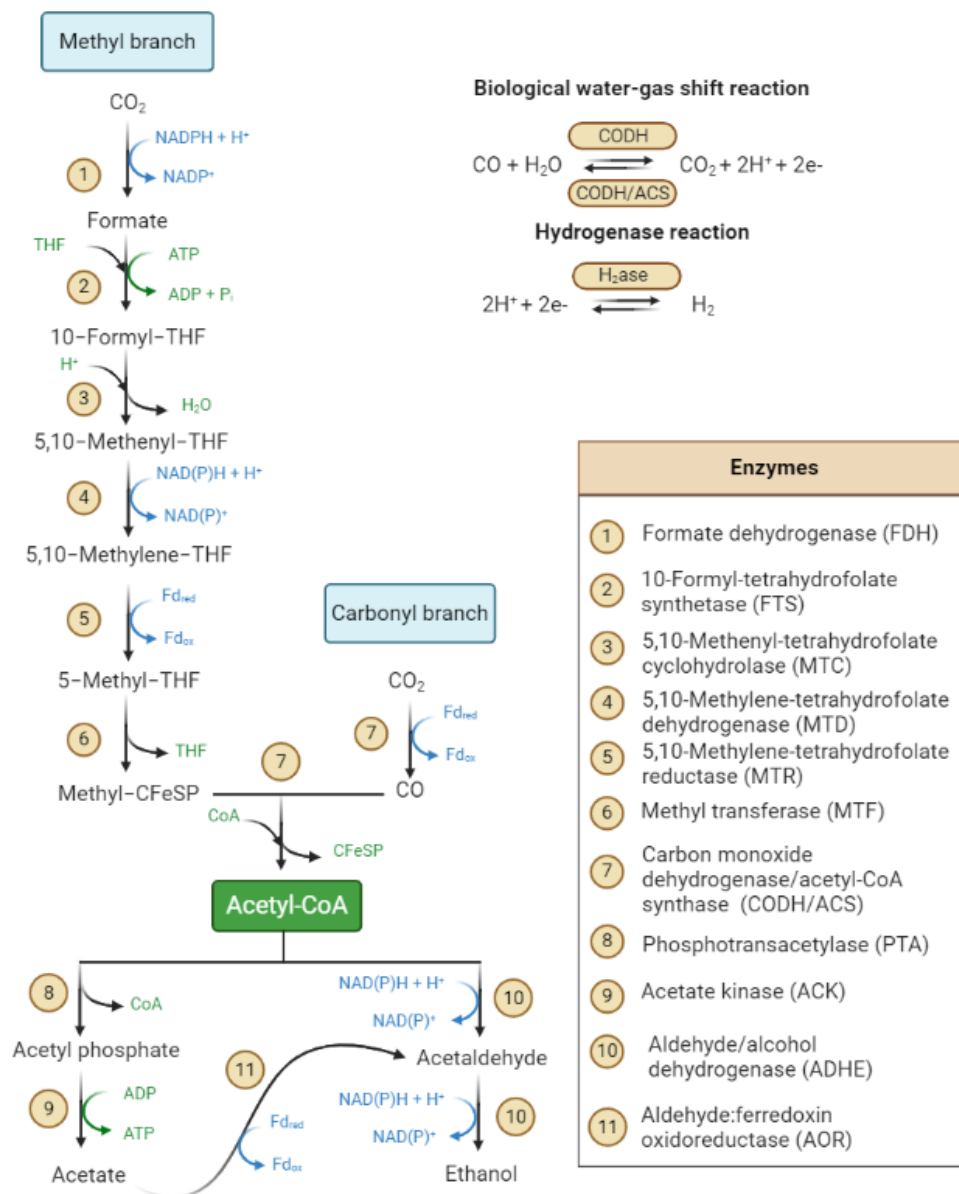
### 3. Syngas Fermentation

#### 3.1. Metabolism Insight of Syngas Fermentation

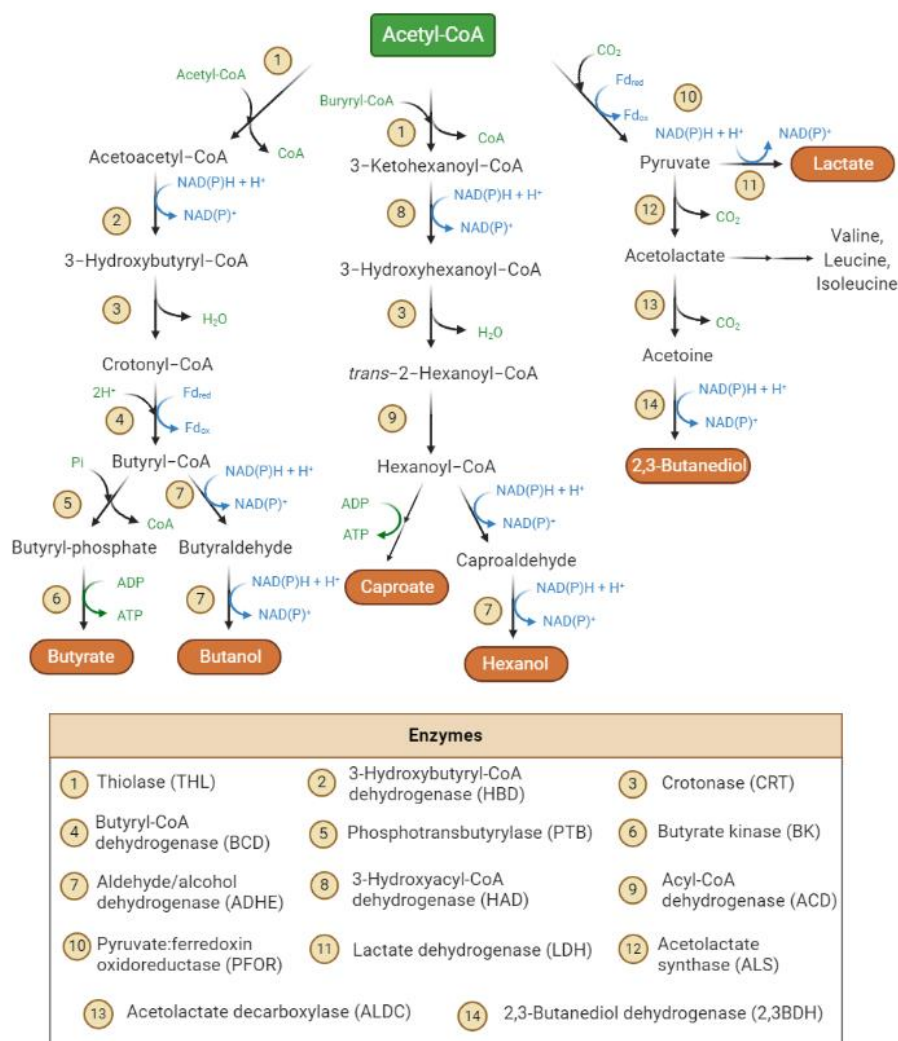
Different types of microorganisms, bacteria, or archaea, whether aerobic or anaerobic, are known to be able to use CO or CO<sub>2</sub> as a carbon and/or energy source. These microorganisms are defined either as autotrophs when they use only C1 compounds as a carbon source and H<sub>2</sub> or light as an energy source, or as unicarbonotrophs when they use C1 compounds as a carbon and energy source. Among these microorganisms, acetogenic bacteria are the most used in syngas fermentation [20,68].

##### 3.1.1. Acetogens and the Wood–Ljungdahl Pathway

Acetogens are obligate anaerobic microorganisms that are able to use CO and/or CO<sub>2</sub> with H<sub>2</sub> to produce organic acids, alcohols, and other industrially relevant chemicals. To date, more than 100 acetogenic species belonging to 22 genera are known, which have been isolated from different habitats. *Acetobacterium* and *Clostridium* are the most representative of these genera. Despite the wide diversity between these microorganisms, the Wood–Ljungdahl metabolic pathway is commonly used [69]. This pathway is a non-photosynthetic metabolic route that assimilates CO<sub>2</sub> into biomass and cell components. It is an irreversible, non-cyclic, strictly anaerobic pathway that consists of two branches: a methyl branch and a carbonyl branch (Figure 4) [20]. Acetogens' metabolism is characterized by two phases. The first phase, known as acidogenesis, is associated with growth. During this phase, microorganisms produce organic acids, mainly acetic acid, in addition to biomass. Acetate production is coupled with the substrate-level phosphorylation (SLP) mechanism, which generates one molecule of ATP per mole of acetate formed. The second phase, which takes place at the stationary growth phase, is named solventogenesis. When microorganisms enter this phase, alcohols are produced from both acetyl-CoA and from organic acids through their reduction. The main product of solventogenesis is ethanol. Therefore, ethanol, unlike acetic acid, is a non-growth-associated product. Solventogenesis is induced by several factors, such as a high concentration of protonated organic acids, non-optimal temperatures, a low pH, a limited concentration of sulfate and phosphate salts, and a high ATP/ADP ratio and/or NAD(P)H levels [70]. During autotrophic growth, reducing equivalents (2H<sup>+</sup> + 2e<sup>-</sup>) can be provided from both CO and H<sub>2</sub> [71]. Before the beginning of the metabolic pathway, 1 mole of CO and 1 mole of H<sub>2</sub>O are converted into 2 moles of reducing equivalents by monofunctional CO-dehydrogenase (CODH) through the biological water–gas shift reaction (Figure 4). Moreover, through the oxidation process catalyzed by hydrogenase (H<sub>2</sub>ase), 1 mole of H<sub>2</sub> is converted into 2 moles of reducing equivalents (Figure 4) [72]. It is important to mention that H<sub>2</sub>ase is CO-sensitive; therefore, H<sub>2</sub> consumption occurs only when CO is completely consumed or its concentration in the medium is low enough [73]. Acetate and ethanol production is possible from any combination of CO and H<sub>2</sub>. Specifically, 4 moles of CO and/or H<sub>2</sub> are used to produce acetate, while 6 moles of CO and/or H<sub>2</sub> are used to produce ethanol [71,74,75]. Therefore, carbon fixation is independent of the origin of the reducing equivalents, although a higher H<sub>2</sub>/CO molar ratio produces an improved CO conversion efficiency into acetic acid and ethanol [69]. Butyric acid, butanol, caproic acid, hexanol, 2,3-butanediol, and lactate can be produced in addition to acetic acid and ethanol (Figure 5).



**Figure 4.** The Wood-Ljungdahl pathway: a schematic representation. THF, tetrahydrofolate; Fd<sub>red</sub>, reduced ferredoxin; Fd<sub>ox</sub>, oxidized ferredoxin; CoA, coenzyme A; CFeSP, corrinoid iron-sulphur-containing protein. Created in BioRender.com.



**Figure 5.** Acetyl-CoA metabolism for the production of butyrate, butanol, caproate, hexanol, 2,3-butanediol, and lactate.  $Fd_{red}$ , reduced ferredoxin;  $Fd_{ox}$ , oxidized ferredoxin; CoA, coenzyme A. Created in BioRender.com.

### 3.1.2. Energy Conservation Model in Acetogens

The ATP production of acetogenic microorganisms is based on the establishment of a chemiosmotic mechanism. Although the SLP mechanism, in which an exergonic chemical reaction is coupled with the phosphorylation of ADP, is used during the Wood–Ljungdahl pathway, no net ATP production is obtained [76]. The chemiosmotic mechanism, instead, consists of the establishment of an electrochemical ion gradient across the cytosolic membrane, which is used by a membrane-bound ATP synthase (ATPase) to produce ATP. This mechanism is coupled with cytosolic reactions of the Wood–Ljungdahl pathway, in which reduced ferredoxin ( $Fd^{2-}$ ) is accumulated. Ferredoxin, which acts as an electron acceptor during the metabolic pathway, is then oxidized by membrane-bound protein complexes, creating a  $H^+$  or  $Na^+$  gradient [77]. Acetogens can be classified into two groups based on the bioenergetics mechanism: the ferredoxin: $NAD^+$  oxidoreductase (Rnf complex)-dependent group and the electron-bifurcating hydrogenase (Ech)-dependent group. The Rnf complex, and then ATPase, can use either protons ( $H^+$ ) or sodium ions ( $Na^+$ ), while Ech uses only  $H^+$ . The membrane-bound Rnf complex is proposed to couple the electron transfer from reduced ferredoxin to  $NAD^+$  with the translocation of  $H^+$  or  $Na^+$  across the cytoplasmic membrane. Instead, the Ech is proposed to couple the electron transfer from reduced ferredoxin to  $NAD^+$  with the translocation of  $H^+$  across the cytoplasmic membrane, with the simultaneous  $H_2$  formation [77].

### 3.2. Syngas Fermenting Microorganisms

#### 3.2.1. Pure Culture Syngas Fermentation

The most commonly used microorganisms in pure culture syngas fermentation are *Acetobacterium woodii* [78], *Clostridium aceticum* [79], *Clostridium autoethanogenum* [80], *Clostridium carboxidivorans* [81], *Clostridium ljungdahlii* [82], and *Clostridium ragsdalei* [83], which were isolated from different habitats, such as marine sediments, soil, animal feces, agricultural settling lagoons, and chicken yard waste. All these microorganisms have a rod-shaped morphology with single, pairs, or chains organization, and are mostly motile due to flagella. The Gram-reaction is positive, except for *C. aceticum* and *C. ljungdahlii*. Moreover, these microorganisms are classified as risk group 1 organisms, and thus, they are suitable for biotechnological applications, with optimal temperatures between 30 °C and 37 °C and an optimal pH range between 5.8 and 6.8, except for *C. aceticum*, whose optimal growth pH is 8.3. Acetogenic microorganisms are obligate anaerobes that are able to use different syngas components (i.e., CO, CO<sub>2</sub>, H<sub>2</sub>) and organic compounds (i.e., sugars, amino acids, alcohols, carboxylic acids, and other substrates) as carbon and energy sources. The main end products are acetic acid and ethanol, but some strains are able to also produce butyric acid, butanol, caproic acid, hexanol, 2,3-butanediol, and lactate (Table 3).

#### 3.2.2. Other Strains

Some other strains have shown the ability to use syngas as a carbon and energy source, including *Morella thermoacetica* [77,83], *Eubacterium limosum* [84], *Butyrifacterium methylotrophicum* [85] and *Clostridium drakei* [81,86]. A brief description of these microorganisms is reported below but they will not be further addressed in this review. *M. thermoacetica* is a thermophilic microorganism that is isolated from horse feces. It was used as a model acetogen to elucidate the Wood–Ljungdahl pathway [77,83]. *E. limosum* and *B. methylotrophicum*, which are closely related to each other, are classified as hazardous and therefore allocated to risk group 2. These strains are autotrophic acetogens that can use H<sub>2</sub>, CO<sub>2</sub>, and CO, and produce acetate, butyrate, ethanol, butanol, and lactate [85,87]. *C. drakei* was isolated from acidic coal-mine pond sediments and it is an acetogen microorganism, an obligate anaerobe, and is able to grow both autotrophically with H<sub>2</sub> plus CO<sub>2</sub> and CO and heterotrophically with many organic substrates. Acetic acid, ethanol, butyrate, and butanol are the end-products of its metabolism. This strain is closely related to *C. carboxidivorans* [81,86].

#### 3.2.3. Mixed-Cultures Syngas Fermentation

Mixed cultures are more suitable for continuous syngas fermentation processes since it expands the products spectrum due to the synergic action and increases the economic sustainability of the process due to less expensive culture nutritional requirements. In addition, microbial diversity allows for a high culture adaptation and high starvation resistance [68,88,89]. Mixed cultures can be obtained from sewage sludge used in wastewater treatment or in anaerobic digestion [89,90], from manure and/or animal feces [91], and from sediments or industrial wastes [69], as shown in several studies. In order to develop a mixed culture that is capable of efficiently using syngas, an enrichment approach based on operating parameters optimization is required. Mixed cultures produce not only acetate and ethanol [92] but also higher alcohols, such as butanol and hexanol [89].

Table 3. Overview of acetogenic microorganisms.

Strain	Gram	pH <sub>opt</sub>	T <sub>opt</sub>	Autotrophic Growth	Heterotrophic Growth	Products	Reference
<i>A. woodii</i>	+	6.8	30 °C	CO, CO <sub>2</sub> , H <sub>2</sub>	fructose, glucose, lactate, glycerate, formic acid, and o-methylated organic compounds	Acetate, EtOH	[78,93]
<i>C. aceticum</i>	–	8.3	30 °C	CO, CO <sub>2</sub> , H <sub>2</sub>	Fructose, ribose, glutamate, fumarate, malate, serine, pyruvate, formic acid, ethylene glycol, and ethanol	Acetate, EtOH	[79,94]
<i>C. autoethanogenum</i>	+	5.8–6.0	37 °C	CO, CO <sub>2</sub> , H <sub>2</sub>	Fructose, xylose, arabinose, rhamnose, glutamate, and pyruvate	Acetate, EtOH, 2,3-butanediol, lactate	[75,80]
<i>C. carboxidivorans</i>	+	6.2	38 °C	CO, CO <sub>2</sub> , H <sub>2</sub>	Ribose, xylose, fructose, glucose, galactose, arabinose, mannose, rhamnose, sucrose, cellobiose, trehalose, melezitose, pectin, starch, cellulose, inositol, mannitol, glycerol, ethanol, propanol, 2-propanol, butanol, citrate, serine, alanine, histidine, glutamate, aspartate, asparagine, casamino acids, betaine, choline, and syringate	Acetate, EtOH, butyrate, butanol, caproate, hexanol	[81,95,96]
<i>C. ljungdahlii</i>	–	6.0	37 °C	CO, CO <sub>2</sub> , H <sub>2</sub>	Fructose, glucose, xylose, arabinose, triose, erythrose, fumarate, formic acid, ethanol, and pyruvate	Acetate, EtOH, 2,3-butanediol, lactate	[82,97]
<i>C. ragsdalei</i>	+	6.3	37 °C	CO, CO <sub>2</sub> , H <sub>2</sub>	Pyruvate, threose, xylose, mannose, fructose, glucose, sucrose, ethanol, 1-propanol, casamino acids, glutamate, serine, choline, and alanine	Acetate, EtOH, butanol, 2,3-butanediol, and lactate	[75,83]

pH<sub>opt</sub>, optimal pH; T<sub>opt</sub>, optimal temperature.

Although mixed-culture fermentation represents a valid alternative in the large-scale production of alcohols in comparison to monocultures, in the enriched culture fermentation, human pathogens could be present, such as *Clostridium difficile* and *Clostridium sordellii*. For this reason, adequate enrichment protocols need to be developed to select and use microorganisms with extreme caution [71]. Instead of enriched mixed cultures, synthetic and defined co-cultures can be developed to overcome human health-related issues. Liu et al. demonstrated synergistic action in a mixed culture formed using *Alkalibaculum bacchi* CP15 (56%), a CO-oxidizing, ethanol-producing acetogen [98], and *Clostridium propionicum* (34%), a microorganism that is able to convert alanine, serine, lactate, and other related compounds into propionate and acetate via the acrylate-CoA pathway [99], with the remaining 10% consisting of four other *Clostridium* strains for the production of acetate, ethanol, propanol, and butanol. Microorganism synergic action can achieve a 60% increase in alcohol production compared with monoculture syngas fermentation with only *A. bacchi* [100].

Diender et al. [101] developed a syngas fermentation process with a synthetic co-culture composed of *C. autoethanogenum* and *C. kluyveri* (1:1) to produce medium-chain fatty acids (MCFAs) and their corresponding alcohols. While *C. autoethanogenum* was able to produce acetic acid and ethanol from the CO present in the syngas, *C. kluyveri* was able to produce MCFAs, such as butyric acid and caproic acid, from ethanol via a reversed  $\beta$ -oxidation pathway. The MCFAs produced were further reduced by the enzymatic activities of *C. autoethanogenum*, thereby producing the corresponding higher alcohols, butanol, and hexanol. Richter et al. [102] carried out a further study with a defined co-culture of two strains, namely, *C. ljungdahlii* and *C. kluyveri*, to produce butanol and hexanol, although at a low specificity, with a low amount of octanol.

### 3.3. Strategies for Improving the Syngas Fermentation Process

The utilization of syngas in microbial fermentation is affected by several factors. In the last few years, intense research has been carried out in order to optimize the conditions of syngas fermentation to improve productivity and yield, as well as to reduce production costs. Research has primarily focused on the medium composition and operating conditions. Several studies have been carried out to investigate the effects of nutritional requirements, such as the organic source, vitamins, trace metals, and reducing agent, as well as temperature, pH, and gaseous substrate partial pressure. A common medium used by syngas fermenting microorganisms contains an organic carbon and nitrogen source, vitamins, mineral salts, and trace metals [72], and it has an appropriate oxidation–reduction potential (ORP) to avoid growth inhibition due to a high redox potential [103]. Media optimization studies have been carried out to ensure economic sustainability in a strain-specific manner [104]. As an organic carbon and nitrogen source, several studies reported the use of yeast extract (YE), which contains a wide range of organic substances, such as amino acids, carbohydrates, and nucleotides, as well as micronutrients, such as vitamins. The YE concentration was investigated on *C. autoethanogenum*, where high concentrations led to enhanced microbial growth with a positive effect on acetic acid production [105,106] and decreased production of more reduced products, such as ethanol. Ethanol production was tested with *C. ljungdahlii*, where traces of YE enhanced its production [107]. The effect of a mixture of proteose peptone, beef extract, YE, and  $\text{NH}_4\text{Cl}$  solution was investigated regarding the maintenance of ethanol and acetate production in non-growing cultures of *C. autoethanogenum* and *C. ljungdahlii*, which showed that an organic nitrogen source is necessary for microbial activity [108]. Recent studies showed no positive effects of vitamin supplementation on cell growth or on product formation, especially ethanol [106,109]. These results could have a huge impact on medium growth formulation because vitamins elimination could decrease the medium cost, and thus, the overall costs. An investigation on the effect of trace metals on ethanol production by *C. ragsdalei* showed that copper ( $\text{Cu}^{2+}$ ) removal and higher nickel ( $\text{Ni}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), tungstate ( $\text{WO}_4^{2-}$ ), and selenate ( $\text{SeO}_4^{2-}$ ) concentrations than those in the reference medium increased ethanol production [110]. The effect of molybdenum ( $\text{Mo}^{4+}$ ) was also studied in syngas fermentation with *C. ragsdalei* [77] and *C. carboxidivorans* [111], with a positive and negative effect on ethanol production,

respectively. *C. autoethanogenum* was used to investigate the presence of tungsten, which increased the ethanol/acetate ratio, as well as the 2,3-butanediol/acetate ratio, and selenium, which partly counteracted the favourable effect of tungsten [109]. A zinc addition increased the expression of genes of alcohol biosynthesis and improved the carbon fixation and CO assimilation and conversion during syngas fermentation of *C. carboxidivorans* [112]. Finally, the iron (Fe) ions concentration in syngas fermentation medium must be higher than other metals due to their function as cofactors of several key enzymes of the acetyl-CoA pathway [111]. Since acetogens are not able to grow in a high-redox-potential environment [113], reducing agents, such as cysteine-HCl with or without sodium sulfide [113] and sodium sulfide ( $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ ) [29], are required to induce a metabolic shift to a solventogenic phase [114] and to enhance ethanol production [105]. Several research studies have identified some alternative medium components. Cottonseed extract (CSE) had a similar chemical composition to the standard medium used for syngas fermentation by *C. ragsdalei*, with more specificity for ethanol production in comparison to the standard medium culture [115]. Corn steep liquor (CSL) is a major byproduct of the corn wet-milling industry. Experimental results obtained from bottle and 7.5 L reactor fermentations using either YE or CSL as a medium supplement showed improved butanol and ethanol production with a higher concentration of CSL [116]. Further studies, however, showed that CSL could be a substitute only for YE, vitamins, and minerals, whereas trace metals, ammonium, and a reducing agent should be added [117]. Remarkably, a CSL medium promoted the production of C4 and C6 alcohols and acids, while a CSE medium supported ethanol formation with *C. ragsdalei* and *C. carboxidivorans* [118]. Finally, biochar was also studied as an alternative medium component, which is a carbon-rich material that is obtained from biomass thermochemical conversion and is able to release alkaline elements and trace metals into liquid media. In the work of Sun et al. [71], syngas fermentation with four types of biochar produced from switchgrass (SGBC), forage sorghum (FSBC), red cedar (RCBC), and poultry litter (PLBC) gasification was investigated in comparison to YE. RCBC and PLBC showed an increase in ethanol production by 16.3% and 58.9%, respectively, compared to the YE medium. Moreover, PLBC enhanced the CO and H<sub>2</sub> consumption by 40% and 69% more than YE, respectively. On the other hand, the operating conditions are of utter importance. Temperature can play an important role due to ethanol production coupled with non-optimal growth conditions. In fact, during the syngas fermentation with *C. carboxidivorans* at the sub-optimal temperature of 25 °C, greater alcohol production was observed [96]. Sub-optimal temperatures prevent “acid crash,” a phenomenon in which the fast organic acid accumulation in the medium causes a small or null alcohol production in the solventogenic phase. Moreover, sub-optimal temperatures could prevent cell flocculation that can occur at 37 °C [119]. The syngas fermentation medium pH has a strong influence on acetogens’ metabolism, especially on the selectivity and distribution of end products. The pH effects on the metabolism shift have been widely investigated in several experiments carried out in the range between 4.5 and 6.8 with *C. ljungdhalii* [108], *C. autoethanogenum* [105,109], and *C. carboxidivorans* [120], and in the range between 6.9 and 8.0 with *C. aceticum* [121]. In order to promote ethanol production rather than acetic acid production, two approaches can be applied: (i) switching the pH from a value of 5.75 to the lower value of 4.75 in the same bioreactor [122] or (ii) separating the two phases of metabolism using two-stage bioreactors with different operating conditions, with the first supporting growth and the second promoting ethanol production [123–125]. In the latter case, the pH values are 5.5 and 6.0 for the first stages, while in the second stages, the pH values are lower, i.e., 4.5, 5.0, and 4.4–4.8. The partial pressure of gaseous substrates has a notable effect on acetogens’ metabolism, thereby influencing the cell growth and product distribution [114]. In particular, the CO partial pressure defines the process efficiency, and thus, the utilization of other syngas components. *C. carboxidivorans* was used to investigate the increase of a gaseous mixture’s partial pressure that was composed of CO and CO<sub>2</sub> during syngas fermentation, which allowed for an increased cell concentration of up to 440%. Moreover, it was highlighted that at higher CO partial pressures, the ethanol concentration increased. In this case, microorganisms were able to use excess electrons supplied by CO to reduce acetate into ethanol [126]. *C. aceticum* was tested at a high CO partial pressure (204.68 kPa), resulting in a high

cell concentration and good CO tolerance in a batch system [127]. Mayer et al. [128] showed a strong CO inhibition on *C. aceticum*, with reduced acetate production. However, high CO partial pressure can have negative effects on enzymes involved in the metabolic pathway since they are sensitive to substrate exposure, such as hydrogenase, as shown by *C. carboxidivorans*, in which the hydrogenase activity was reduced by 97% at a CO partial pressure of 202.7 kPa [24]. On the other hand, the increase of the H<sub>2</sub> partial pressure up to 170 kPa produced an increase in the productivity of acetate by *A. woodii* (7.4 g/L/day) [129]. A syngas fermentation in a batch using *C. ljungdahlii* as the biocatalyst was carried out to investigate the syngas total pressure effect without growth inhibition at high values [130]. Moreover, increased ethanol production was observed due to the simultaneous use of CO, CO<sub>2</sub>, and H<sub>2</sub>.

#### 3.4. Mass Transfer Limitations and Bioreactor Optimization

One of the limiting factors in syngas fermentation is the mass transfer [131]; therefore, the choice of bioreactor is a key point to ensure a high cell concentration and productivity [114]. The most commonly used bioreactors (Table 4) are the continuous stirred tank reactor (CSTR), bubble column reactor (BCR), trickle-bed reactor (TBR), membrane bioreactor (MBR), and monolithic biofilm reactor (MLBR) (Figure 6). Several studies were carried out using CSTRs as bioreactors for syngas fermentation using different biocatalysts. *C. ljungdahlii* was used for ethanol and acetate production [132–135] at the laboratory scale with improved performances compared to CO fermentation. *C. carboxidivorans* was studied in CSTRs showing a shifting metabolism to a solventogenic phase with the production of butanol, as well as ethanol [136,137]. The highest ethanol and acetate productions were observed using *C. autoethanogenum* as a biocatalyst [138]. *C. ragsdalei* was studied in a CSTR with 10 g/L poultry litter biochar (PLBC), showing the feasibility of using a PLBC medium to enhance ethanol production from syngas for potential use at a commercial scale [139]. *C. ragsdalei* syngas fermentation was validated in a 100 L pilot-scale CSTR [140], showing a six-fold improvement in ethanol concentration compared to a serum bottle. A bubble column reactor (BCR) ensured an increased volumetric mass-transfer coefficient using a gas sparging system, which makes BCR more suitable for large-scale syngas fermentation than CSTR [21,141,142]. *C. carboxidivorans* provided a higher ethanol selectivity with respect to CO fermentation [142,143]. In a trickle-bed reactor (TBR) configuration, syngas is allowed to move either co-currently or counter-currently to the liquid medium with free or immobilized cells on the bed [21,141]. In a gas-continuous mode, there is a low mass transfer resistance, and thus, it is possible to operate with a low gas flow, thereby achieving a high volumetric mass transfer coefficient and high conversion efficiency. In continuous-liquid mode, instead, it is necessary to implement a gas recirculation system to achieve high conversion efficiency [20]. TBC is more advantageous than CSTR and BCR due to its high conversion rate of syngas and high productivity [105]. *C. ljungdahlii* was studied for acetic acid and ethanol production, while *C. aceticum* was investigated only for acetate production [144–146]. Membrane bioreactor (MBR) configurations with different types of membranes were developed [114], where the hollow-fiber membrane bioreactor (HFMBR) was the most commonly used [21,147]. In particular, this configuration with *C. ragsdalei* [148] was patented for an ethanol production process [149]. *C. ljungdahlii* [150], *C. carboxidivorans* [151], and *A. woodii* [152] have been studied with an HFM configuration with improved ethanol and acetate productivity. Monolithic biofilm reactor (MBR) configuration showed improved mass transfer characteristics [21,153], where *C. carboxidivorans* syngas fermentation obtained a higher ethanol productivity and final titer than other reactors [154].



**Table 4.** Syngas fermentation performance carried out in various reactor configurations.

Reactor Configuration	Syngas Composition	Operative Conditions	Microorganism	Products	Products Concentration-Productivity	Reference
CSTR	19% CO, 77% H <sub>2</sub> , 4% CH <sub>4</sub>	T: 38 °C pH: 5.0 Agitation rate: 1000 rpm	<i>C. ljungdahlii</i>	Ethanol	10.00 g/L 6.70 g/L/day	[134]
CSTR (2 L)	55% CO, 20% H <sub>2</sub> , 10% CO <sub>2</sub> , 15% Ar	T: 37 °C pHi: 6.8 Agitation rate: 500 rpm GFR: 14 mL/min K <sub>L</sub> a: 135 h <sup>-1</sup>	<i>C. ljungdahlii</i>	Ethanol Acetate	6.50 g/L 5.43 g/L	[132,133]
CSTR (3 L)	60% CO, 35% H <sub>2</sub> , 5% CO <sub>2</sub>	T: 37 °C pH: 4.0–4.8 Agitation rate: 300–500 rpm GFR: 5–15 mL/min LFR: 0.25–0.75 mL/min K <sub>L</sub> a: 34.02 h <sup>-1</sup>	<i>C. ljungdahlii</i>	Ethanol Acetate	3.75 g/L 14.97 g/L	[135]
CSTR (7.5 L)	16.5% CO, 15.5% CO <sub>2</sub> , 5% H <sub>2</sub> , 56% N <sub>2</sub>	T: 37 °C pHi: 5.9 to pHf: 5.3 Agitation rate: 400 rpm	<i>C. carboxidivorans</i>	Ethanol Acetate	N.A. N.A.	[136]
CSTR (7.5 L)	20% CO, 5% H <sub>2</sub> , 15% CO <sub>2</sub> , 60% N <sub>2</sub>	T: 37 °C pHi: 5.7 Agitation rate: 150 rpm GFR: 10 standard L/min	<i>C. carboxidivorans</i>	Ethanol Butanol	1.48–2.82 g/L 35.00–65.00 mM/g of cells/day 0.33–0.53 g/L	[137]
CSTR	2% CO, 65% H <sub>2</sub> , 23% CO <sub>2</sub> , 10% Ar	T: 37 °C pH: 5.0 Agitation rate: 800 rpm GFR: 30 mL/min D: 0.5 day <sup>-1</sup>	<i>C. autoethanogenum</i>	Ethanol Acetate	9.69 g/L 5.97 g/L	[138]
CSTR (3 L)	40% CO, 30% H <sub>2</sub> , 30% CO <sub>2</sub>	T: 37 °C pHi: 5.9–4.8 Agitation rate: 200–300 rpm GFR: 1.3–1.8 mmol/min	<i>C. ragsdalei</i>	Ethanol	11.00 g/L	[139]

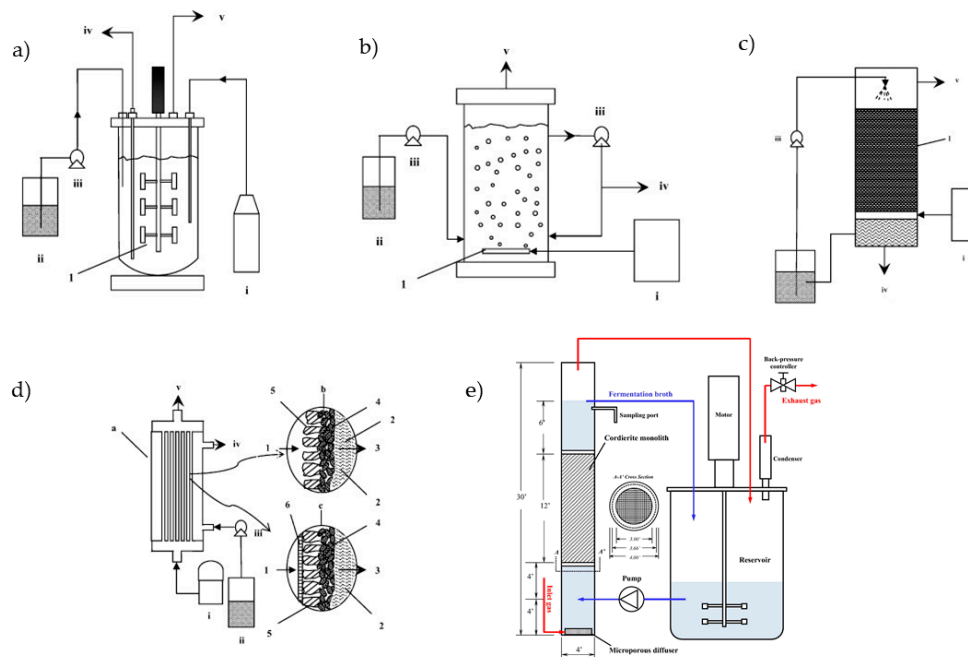
Table 4. Cont.

Reactor Configuration	Syngas Composition	Operative Conditions	Microorganism	Products	Products Concentration-Productivity	Reference
<b>CSTR (100 L)</b>	5% H <sub>2</sub> , 15% CO <sub>2</sub> , 20% CO, 60% N <sub>2</sub>	T: 37 °C pHi: 5.9 to pHf: 4.7 Agitation rate: 150 rpm GFR: 0.9 standard L/min	<i>C. ragsdalei</i>	Ethanol Acetate 2-Propanol 1-Butanol	25.26 g/L 4.82 g/L 8.86 g/L 0.47 g/L	[140]
<b>BCR (4.5 L)</b>	25% CO, 15% CO <sub>2</sub> , 60% N <sub>2</sub>	T: 37 °C pH: 5.8 GFR: 200 ccm LFR: 200–300 mL/min D: 0.026 h <sup>-1</sup>	<i>C. carboxidivorans</i>	Ethanol Acetate Butanol	N.A. N.A. N.A.	[143]
<b>BCR (4 L)</b>	14.7% CO, 4.4% H <sub>2</sub> , 16.5% CO <sub>2</sub> , 56.8% N <sub>2</sub> , 4.2% CH <sub>2</sub> , 2.4% C <sub>2</sub> H <sub>4</sub> , 0.8% C <sub>2</sub> H <sub>6</sub>	T: 37 °C pHi: 5.8–5.9 GFR: 180 ccm LFR: 1.5 mL/min	<i>C. carboxidivorans</i>	Ethanol Acetate Butanol Butyrate	1.60 g/L N.A. N.A. N.A.	[142]
<b>TBR (1 L)</b>	38% CO, 28.5% H <sub>2</sub> , 28.5% CO <sub>2</sub> , 5% N <sub>2</sub>	T: 37 °C pH: 5.8 GFR: 2.3 or 4.6 sccm LFR: 200, 500, 700 mL/min (semi-continuous mode)	<i>C. ragsdalei</i>	Ethanol Acetate	5.70 g/L and 37.00 mg/L/h 12.30 g/L	[144]
<b>TBR (1 L)</b>	38% CO, 28.5% H <sub>2</sub> , 28.5% CO <sub>2</sub> , 5% N <sub>2</sub>	T: 37 °C pHi: 5.8 GFR: 2.8–18.9 sccm LFR: 200–500 mL/min D: 0.012 h <sup>-1</sup> (continuous mode)	<i>C. ragsdalei</i>	Ethanol Acetate	13.20 g/L and 158.00 mg/L/h 3.30 g/L	[155]
<b>HFMBR</b>	40% CO, 30% H <sub>2</sub> , 30% CO <sub>2</sub>	T: 37 °C pHi: 5.9 to pHf: 4.5 Agitation rate: 100 rpm GFR: 60 std L/min LFR: 180 mL/min	<i>C. ragsdalei</i>	Ethanol	10.00 g/L	[149]
<b>HFMBR</b>	50% CO, 30% H <sub>2</sub> , 20% CO <sub>2</sub>	T: 35 °C pH: 5.0 GFR: 250 mL/min K <sub>L</sub> a: 385.2 h <sup>-1</sup>	<i>C. ljungdahlii</i>	Ethanol Acetate	6.00 g/L 3.00 g/L	[150]

Table 4. Cont.

Reactor Configuration	Syngas Composition	Operative Conditions	Microorganism	Products	Products Concentration-Productivity	Reference
<b>HFMBR (8 L)</b>	20% CO, 5% H <sub>2</sub> , 15% CO <sub>2</sub> , 60% N <sub>2</sub>	T: 37 °C pHi: 6.0 to pHf: 4.5–5.5 Agitation rate: 200 rpm GFR: 50–300 mL/min LFR: 50–200 mL/min K <sub>L</sub> a: 1096.2 h <sup>-1</sup>	<i>C. carboxidivorans</i>	Ethanol Acetate	23.93 g/L and 3.44 g/L/day 5.00 g/L	[151]
<b>CSTR (1 L) with a submerged HFMs module</b>	40% H <sub>2</sub> , 17% CO <sub>2</sub> , 43% N <sub>2</sub>	T: 30 °C pH: 7.0 Agitation rate: 1200 rpm GFR: 30 L/h D: 0.35 h <sup>-1</sup>	<i>A. woodii</i>	Acetate	17.60 g/L and 148.00 g/L/day	[152]
<b>MLBR (8 L)</b>	20% CO, 5% H <sub>2</sub> , 15% CO <sub>2</sub> , 60% N <sub>2</sub>	T: 37 °C pHi: 6.0 to pHf: 4.5–5.5 GFR: 50–300 mL/min LFR: 200–500 mL/min D: 0.48 day <sup>-1</sup> K <sub>L</sub> a: 50–550 h <sup>-1</sup>	<i>C. carboxidivorans</i>	Ethanol Acetate	4.89 g/L and 2.35 g/L/day 3.05 g/L and 1.46 g/L/day	[154]

GFR, gas flow rate; LFR, liquid flow rate; D, dilution rate; K<sub>L</sub>a, volumetric mass transfer coefficient; pHi, initial pH; pHf, final pH.



**Figure 6.** Schematic illustrations of common bioreactors for syngas fermentation: (a) continuous stirred tank reactor (CSTR), (b) bubble column reactor (BCR), (c) trickle-bed reactor (TBR), (d) hollow-fiber membrane bioreactor (HFMBR) ([114]; permission obtained by Wiley Online Library), and (e) MLBR ([154]; permission obtained by Elsevier).

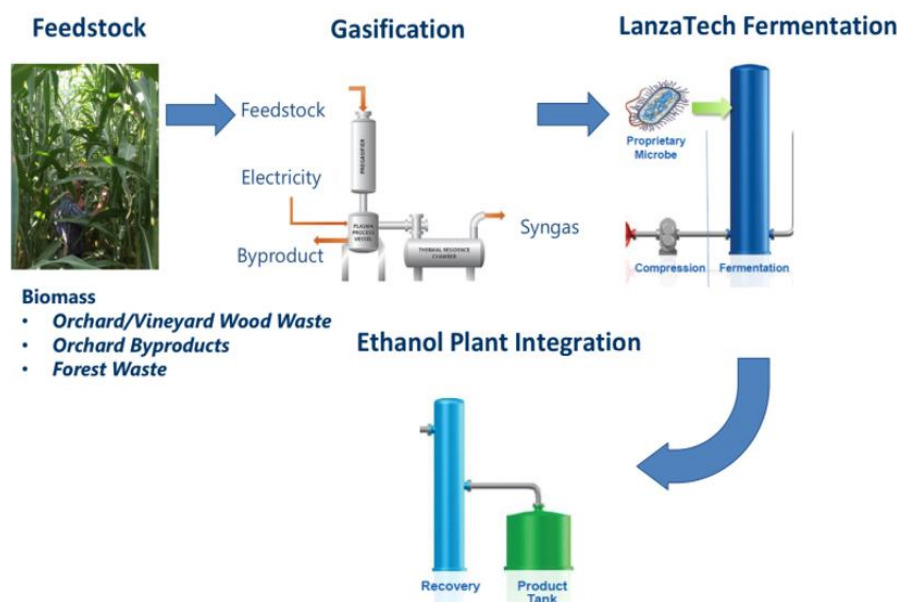
#### 4. Biomass Gasification and Syngas Fermentation at a Large Scale

The gasification of biomass followed by syngas fermentation to produce fuels and commodity chemicals is a developing technology. Nowadays, LanzaTech Inc. is the leading company regarding the syngas fermentation process. Founded in 2005, LanzaTech Inc. has developed a technology for the use of syngas and industrial waste gases to produce ethanol and 2,3-butanediol using a proprietary recombinant microorganism, i.e., *C. autoethanogenum*. LanzaTech Inc. holds a portfolio of over 100 patents that are related to genetic manipulation to enhance products of interest, reactor designs, process developments to enhance mass transfer and optimize operating conditions, process controls for stable operation and increased selectivity and productivity, syngas clean-up strategies, and product recovery technologies [156,157]. Nowadays, LanzaTech has four demonstration facilities using steel mill off-gases for the production of ethanol in New Zealand, China, and Taiwan. In particular, the two pre-commercial demonstration plants in China, built in partnership with BaoSteel and Shougang, the two major Chinese steel companies, have an ethanol production capacity of 300 metric tons/year each [157]. Moreover, the company is building a commercial plant with an ethanol production capacity of 62,000 metric tons/year in Belgium in partnership with the steel producer ArcelorMittal.

The patent specifications indicate the use of a loop reactor by LanzaTech Inc. for the syngas fermentation process. In a 2012 U.S. patent, a loop reactor was described for the steel mill off-gas (47% CO, 2% H<sub>2</sub>, 21% CO<sub>2</sub>, 30% N<sub>2</sub>) fermentation using *C. autoethanogenum* DSMZ 19630 as the biocatalyst. This configuration consists of a 71 L vessel, a gas inlet, a gas outlet, and an external liquid pump for the circulation of the liquid phase. A vessel section is filled with Sulzer Mellapak™ structured packing, which acts as a contact module between the gas and liquid phases. The process was carried out at a temperature of 37 °C and a pH of 5.3, with a centrifugal pump rate of 600–1250 m<sup>3</sup>/h. The gas–liquid mass transfer was improved, achieving a volumetric mass transfer coefficient ( $K_L a$ ) of 0.14 s<sup>-1</sup> and a CO uptake of 5.66 mol/L/day. The final ethanol production was 25 g/L with a productivity of 23.9 g/L/h [158]. Moreover, the gas–liquid mass transfer was improved in forced circulating bubble column reactors by introducing a secondary circulating loop. This configuration consists of a vessel comprising two sections: a riser section (80% of the total reactor volume), wherein the liquid medium

and the syngas move concurrently upward, and a downcomer section, wherein the liquid medium and the syngas move downward. The vessel has a volume of either 390 L (height: 6 m) or 9800 L (height: 10 m). The liquid medium and the syngas are circulated with an external pump in a primary loop. An additional recirculation loop leads to improved gas conversion. The liquid flow rate in the downcomer section was 30 m<sup>3</sup>/h, and in the second loop, it was 5.5 m<sup>3</sup>/h. This configuration increased the gas retention in the reactor and avoided foaming issues during fermentation [159].

The process shows high feedstock flexibility, thereby allowing for the use of both industrial waste gases and syngas produced through the gasification of biomass wastes. In 2018, LanzaTech Inc. (Skokie, IL, USA) established a partnership with Aemetis, Inc. (Cupertino, CA, USA), which is an advanced renewable fuels and biochemicals company focused on the acquisition, development, and commercialization of innovative technologies that replace traditional petroleum-based products via the conversion of ethanol and biodiesel plants into advanced biorefineries. Aemetis, Inc. successfully developed an integrated demonstration unit for the hybrid process based on biomass gasification and syngas fermentation. The demonstration plant used waste orchard wood and almond and walnut shells as feedstock for the high-temperature plasma gasification system to produce syngas. Then, the cooled and cleaned syngas was supplied to a patented syngas fermentation bioreactor from LanzaTech Inc. to produce cellulosic ethanol. The broth was subsequently distilled to produce commercial-grade ethanol (Figure 7) [160]. Located at InEnTec's Technology Center in Richland, Washington, the plant was continuously operated for more than 120 days with a 94% uptime. This showed that Aemetis, Inc. can successfully produce high-value ethanol from waste orchard wood and other renewable feedstocks, developing a full-scale operating biorefinery [160]. Aemetis, Inc. planned to build a commercial plant in Riverbank, California, to produce 12 billion gallons per year of cellulosic ethanol from 1.6 million tons of waste orchard wood and other renewable feedstocks generated in the California Central Valley [160].



**Figure 7.** Schematic representation of the demonstrative plant developed by Aemetis, Inc. for ethanol production from syngas [160].

## 5. Future Perspectives on Strategies for a Sustainable Biomass-Derived Syngas Valorization

Biorefinery systems based on biomass gasification and syngas fermentation need further investigation, both to overcome challenges and increase its commercial interest. One of the major challenges affecting the integration of biomass gasification and syngas fermentation is the syngas final quality. Therefore, choosing the most suitable syngas clean-up strategy to remove impurities is a

key element in the development of this biorefinery system on a large scale. Moreover, so far, syngas fermentation has been studied for the production of a limited range of chemicals, mostly ethanol and acetate. In order to extend the target market of syngas-based biorefinery, further studies are required, both to identify new non-acetogenic syngas-fermenting microorganisms and to develop cascade approaches in which syngas fermentation is coupled with other bioprocesses.

### 5.1. Effects of Syngas Impurities and Syngas Clean-Up

Nowadays, gasification–fermentation research is commonly performed at the bench scale with clean syngas instead of biomass-derived syngas. The major difference between syngas produced from biomass gasification (“raw syngas”) and purified, bottled, mixed commercial syngas (“clean syngas”) is the presence of impurities and residues in addition to the main components (CO, H<sub>2</sub>, and CO<sub>2</sub>). Understanding of effects of syngas impurities on the fermentation process is critical for the design of an economically viable gasification–fermentation process, based on the development of syngas clean-up strategies [31].

Some impurities, such as light hydrocarbons, did not show any influence on the growth or the metabolism of *C. carboxidivorans* at concentrations below 5% [136,142]. Moreover, acetogens tolerate up to a 20% sulfur content, mainly in the form of hydrogen sulfide (H<sub>2</sub>S) and carbonyl sulfide (COS) in syngas, which is useful during fermentation due to the reduction of the redox potential and removal of O<sub>2</sub> from the medium [37]. On the other hand, regarding the effects of tars, the properties and composition of the aromatic mixture are relevant rather than the quantity [161]. In particular, benzene, naphthalene, and polyaromatic hydrocarbons (PAHs) have an adverse effect on cell growth, as well as on hydrogenase activity due to their high solubility in the medium during interactions with microorganisms [31,37]. Tars can induce a steady or dormant state with a lack of H<sub>2</sub> uptake, followed by a metabolic shift from an acidogenesis to a solventogenesis phase, with higher ethanol production compared to acetate [136,142]. Similarly, nitrogenous species, such as nitric oxide (NO) and ammonia (NH<sub>3</sub>), and thus, ammonium ions (NH<sub>4</sub><sup>+</sup>), at concentrations of 0.4–0.15 g/L, 37% molar fraction, and 6.84 g/L, respectively, are enzyme inhibitors, especially ammonia for alcohol dehydrogenase and ammonium ions and nitric oxide for hydrogenase [162,163]. Interestingly, the presence of hydrogen cyanide (HCN) prolonged the lag phase of *C. ljungdahlii* [164].

There are different technologies for syngas clean-up (Table 5). The most suitable technology is dependent on affordability, environmental impact, and the final syngas quality required. Physical methods, such as cyclone, electrostatic precipitator (ESP), rotating particle separator (RPS), and filtration methods, are the simplest and the most mature technologies, but they are not suitable for tar removal due to the presence of very small particles [165]. Wet scrubbing technologies are effective in removing tars, as well as other impurities (i.e., NH<sub>3</sub>, HCl). However, this technology needs additional steps, such as syngas cooling and wastewater treatment, which increase the overall energy input and costs [166]. Tars’ thermal cracking is difficult to control due to the high process and equipment complexity and the process being highly dependent on many parameters [18,161]. The utilization of catalysts for hot gas cleaning is one of the most efficient technologies for impurities removal (i.e., tars, NH<sub>3</sub>, NO<sub>x</sub>, N<sub>2</sub>O, H<sub>2</sub>S) and the most viable option for the large-scale production of clean syngas [165]. Mineral-based catalysts are natural inexpensive minerals, including calcined rocks (calcined dolomite, magnesite, and calcite), olivine, clay materials, and iron ores. On the other hand, synthetic catalysts are char, alkali metal-based, activated alumina, and transition metal-based (Pt, Zr, Rh, Ru, Fe, and Ni) catalysts. Transition metals, as well as other synthetic catalysts, are relatively more expensive compared to the mineral catalysts, although they are more effective, especially for tar reduction [165,166]. However, synthetic catalysts undergo several deactivation mechanisms due to sulfur, chlorine, and alkali metals, which are present at high levels in biomass [167]. Further investigations are required to analyze the catalysts’ performance, life cycle, costs and benefits, and techno-economic feasibility.

**Table 5.** Overview of syngas clean-up technologies.

Methods	Brief Description	Impurities Removed	Reference	
<b>Physical methods</b>	Cyclone separators	A centrifugal force is applied to separate solids and aerosols from the gas.	Large particles (>5 $\mu\text{m}$ diameter), tars	[161,166,168,169]
	Electrostatic precipitators (ESPs)	Dust particles and droplets of tar attach to gas ions produced in a corona discharge. Particles and droplets become charged and precipitate due to an applied electric field.	Particulate <sup>a</sup> , tars	[161,166,168,169]
	Rotating particle separators (RPSs)	It is made of a rotating cylinder, where (i) tars are condensed and then the droplets are removed or (ii) a solvent is injected and then the saturated solvent is taken out.	Particulate <sup>a</sup> , tars	[161]
	Filtration	Tars, dust, and particles are blocked on the filter surface. Different types of filters can be used, such as fabric filters, ceramic filters, activated carbon-based absorbers, sandbed filters, and catalytic filters.	Particulate <sup>a</sup> (tar elimination is not efficient)	[161,168,169]
	Wet scrubber (water, RME, OLGA scrubber <sup>b</sup> )	It is composed of a gas cooler, fine tar mist separator, and occasionally, a solid particle separator. Impurities elimination occurs via condensation, precipitation, diffusion, solubility, and absorption.	Tars, NH <sub>3</sub> , HCl	[166,169]
	Rectisol wash	This process takes place at a temperature below $-40\text{ }^{\circ}\text{C}$ and at high pressure. Methanol is used as a solvent due to its ability to allow for physical absorption of NH <sub>3</sub> and HCN, but also of H <sub>2</sub> S and CO <sub>2</sub> .	NH <sub>3</sub> , HCN, H <sub>2</sub> S, and CO <sub>2</sub>	[168,169]
	Absorption	It is a physical phenomenon consisting of the penetration of a substance into a solid (polyethylene glycol; oxides of Fe, Mn, Zn, Cu, and Ca; molecular sieves) or liquid body (alkaline solution).	H <sub>2</sub> S	[168,169]
	Membrane permeation	It is the separation of individual compounds on the basis of the difference in their rates of permeation through a thin membrane barrier.	H <sub>2</sub> S	[170]
<b>Thermal methods</b>	Thermal cracking	It consists of a high-temperature (>1000 $^{\circ}\text{C}$ ) treatment, during which heavy tar compounds are decomposed into lighter compounds.	Tars	[161,168,169]
<b>Catalytic methods</b>	Hot catalytic gas conditioning	Catalytic strategies provide the possibility to transform the impurities into useful gas compounds, such as CO and H <sub>2</sub> . Catalysts can be natural minerals (calcined rocks, such as calcined dolomite, magnesite, and calcite; olivine; clay materials; iron ores) or metallic and metal oxide synthetic catalysts (alkali metal-based, activated alumina, and transition metal-based catalysts, such as Pt, Zr, Rh, Ru, Fe, and Ni).	Tars, NH <sub>3</sub> , NO <sub>x</sub> , N <sub>2</sub> O, H <sub>2</sub> S	[161,168,169]

<sup>a</sup> Particulates include char, ash, and alkalis present in the syngas. <sup>b</sup> A wet scrubber can use different liquids, such as water, rapeseed oil methyl ester (RME), or oil washing medium (OLGA).

## 5.2. Cascade Approaches

Coupling syngas fermentation with other bioprocesses can allow for the expansion of syngas fermentation products and improve product yields and economics. Coupled processes are defined as multistep bioprocesses that convert metabolites from a primary fermentation into valuable products in a secondary fermentation [103]. Acetic acid and ethanol, which are the main products of syngas fermentation, can be used as a substrate to produce C4–C6 carboxylic acids and their corresponding alcohols [171], dicarboxylic acids [172], lipids [51], and polyhydroxyalkanoates (PHAs) [173] (Table 6).

### 5.2.1. Syngas into Elongated Carboxylic Acids

Products from syngas fermentation can be converted by microorganisms other than acetogens into elongated carboxylic acids. Moreover, these carboxylic acids can be reduced into their corresponding alcohols using acetogens. Indeed, acetogens are able to reduce carboxylic acids first into the corresponding aldehydes and then into the corresponding alcohols via the aldehyde:ferredoxin oxidoreductase (AOR) and the aldehyde/alcohol dehydrogenase (ADHE) activity, respectively [104].

It has been demonstrated that some acetogens, such as *C. ljungdahlii* and *C. ragsdalei*, use syngas as a source of electrons and energy to reduce externally added acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and caproic acid into their corresponding alcohols [174,175].

These alcohols are more energy-dense and easier to recover than ethanol; thus, they show great potentiality, both as drop-in fuels and as valuable commodity chemicals [104].

A proof-of-concept system in which syngas fermentation was coupled with a chain elongation process to avoid cost-intensive ethanol recovery step was shown [171]. Effluent from a syngas fermenting system with a pure culture of a *C. ljungdahlii* strain was used as a liquid feed medium for the chain elongation bioreactor. The syngas used in the first phase consisted of a gas mixture of 65% CO, 30% H<sub>2</sub>, and 5% CO<sub>2</sub>, and it was converted into 11.4 g/L of ethanol and 2.3 g/L of acetic acid. This effluent (2–4% w/w metabolite concentration) was used as a liquid medium in an anaerobic reactor with an open mixed culture. Ethanol was converted into caproic acid via chain elongation via the reversed  $\beta$ -oxidation pathway.

### 5.2.2. Syngas into Dicarboxylic Acids

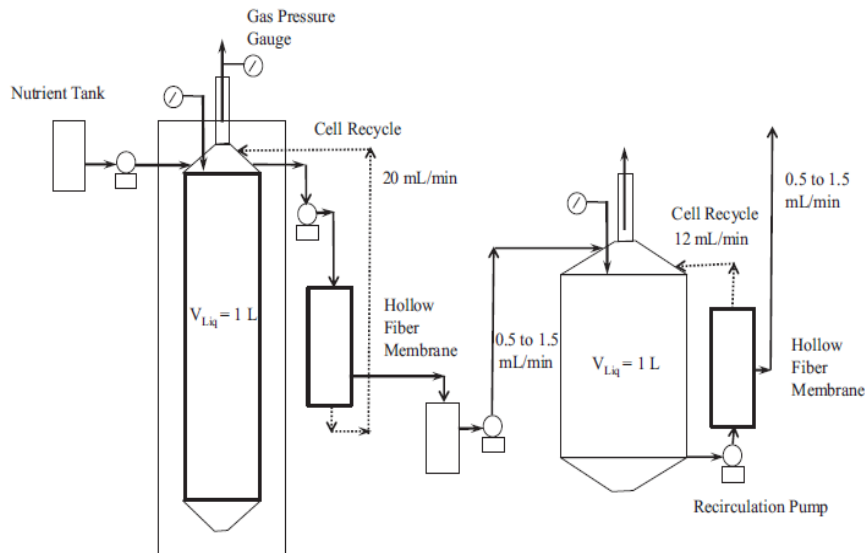
Malic acid, together with fumaric and succinic acids, was selected by the United States Department of Energy to be one of the 12 most important platform chemicals produced from biomass. Malic acid can be used for the synthesis of polymers and for the food and pharmaceutical industries, as well as for many other bulk and fine chemicals. An integrated process in which acetic acid from syngas fermentation was converted into malic acid was developed [172]. Sequential production of malic acid from syngas took place in two different 2.5 L CSTRs. For the first step, *C. ljungdahlii* was used as a biocatalyst during syngas fermentation. The composition of syngas used inside the reactor was 32.5% H<sub>2</sub>, 32.5% CO, 16% CO<sub>2</sub>, and 19% N<sub>2</sub>. This process took place under controlled operational conditions: a temperature of 37 °C, pH of 5.9, gas flow rate of 20 mL/min, and agitation speed of 800 rpm. During the 96 h process, *C. ljungdahlii* produced both acetate and ethanol, with final concentrations of 15.9 g/L and 2.0 g/L, respectively. Broth from the syngas fermentation was used for sequential fermentation conducted by *Aspergillus oryzae*. This fungal species was able to transform acetic acid into malic acid at a concentration of 1.83 g/L. The overall yield for the conversion of syngas in malic acid was 0.22 g/g (3.5 g malic acid per mol of syngas) [172].

### 5.2.3. Syngas into Lipids

Lipids are the main platform for the production of biodiesel, which is one of the most promising clean and renewable liquid fuels. Nowadays, lipids are produced from carbohydrate feedstocks via specialized crops or oleaginous microorganisms. An alternative gas-to-lipids approach that overcomes the limits of conventional processes was reported [51].



This approach was characterized by two stages. During the first stage, syngas was converted by the thermophilic acetogen *M. thermoacetica* into acetic acid. The acetic acid product was then fed as a substrate into a second bioreactor, where it was converted into lipids by the engineered oleaginous yeast *Yarrowia lipolytica*. The two stages were integrated into a single continuous-flow system (Figure 8), which consisted of an anaerobic BCR for the first stage and an aerobic CSTR for the second stage.



**Figure 8.** Schematic representation of a two-stage system for the production of lipids from syngas [51].

#### 5.2.4. Syngas into Polyhydroxyalkanoates

PHAs are promising bioplastics due to having similar physico-chemical properties as conventional plastics. Several microorganisms are able to produce PHA, where the greatest yield is obtained using a pure culture of bacteria or archaea species. However, this bioprocess is not competitive with the fossil-based one due to high costs. A more convenient alternative is represented by the use of mixed microbial cultures, as well as cheaper substrates. Lagoa-Costa et al. [173] proposed an integrated two-stage bioconversion process for both bioethanol and PHA production. In the first stage, *C. autoethanogenum* carried out the anaerobic bioconversion of syngas, which consisted of a gas mixture of 30% CO, 10% CO<sub>2</sub>, 20% H<sub>2</sub>, and 40% N<sub>2</sub> in a 2 L CSTR. The final ethanol concentration was 3.79 g/L, while the acetic acid concentration dropped to 2.66 g/L. This effluent was used as a substrate to produce PHA in a fermentation process, which took place in a glass bioreactor filled with a mixed microbial culture (MMC). The second stage was operated in fed-batch mode. The maximum amount of PHA of 24% of the cell dry weight, in the form of polyhydroxybutyrate (PHB), was produced. Since acetic acid was only used as a substrate to produce PHA, alcohols such as ethanol could be recovered before the second stage. Moreover, a two-stage, whole-cell biocatalytic system for producing PHB from carbon monoxide via formate as an intermediate was developed [176]. In the first stage, *A. woodii* converted a different syngas mixture (20–65% CO, 2–14% H<sub>2</sub>, 16–42% CO<sub>2</sub>, 18–56% N<sub>2</sub>) into a single final product, i.e., formate, achieving high selectivity due to the suppression of acetate production by removing Na<sup>+</sup>. Formate was then supplied to a 1 L fermenter in which a genetically modified *Methylobacterium extorquens* strain converted it into PHB, with a concentration and cell contents of 0.097 g/L and 6.5%, respectively. The overall yield of PHB from CO was 2.24%.

Table 6. Cascade approaches overview.

• Syngas into Elongated Carboxylic Acids						
Stage 1:	Microorganism	Substrate	Operative Conditions	Reactor	Products	Ref.
Syngas fermentation	<i>C. ljungdahliae</i>	65% CO <sub>2</sub> , 30% H <sub>2</sub> , 5% CO <sub>2</sub>	N.A.	N.A.	Ethanol (11.4 g/L) Acetate (2.3 g/L)	[171]
Stage 2: Chain elongation process	Mixed culture	Ethanol	T: 30 °C pHi: 6.5 to pHf: 5.5	Anaerobic filter reactor (700 mL)	Acetic acid (7.9 g/L) Butyric acid (19.4 g/L) Caproic acid (1.0 g/L)	
• Syngas into Dicarboxylic Acids						
Stage 1: Syngas fermentation	<i>C. ljungdahliae</i>	32.5% H <sub>2</sub> , 32.5% CO <sub>2</sub> , 16% CO <sub>2</sub> , 19% N <sub>2</sub>	T: 37 °C pH: 5.9 GFR: 0.02 L/min Agitation speed: 800 rpm	CSTR (2.5 L)	Ethanol (2.0 g/L) Acetate (15.9 g/L)	[172]
Stage 2: Malic acid production	<i>Aspergillus oryzae</i>	Acetic acid	T: 35 °C pH: 6.5 Aeration rate: 0.6 L/min Agitation speed: 300 rpm	CSTR (2.5 L)	Malic acid (1.8 g/L)	
• Syngas into Lipids						
Stage 1: Syngas fermentation	<i>M. thermoacetica</i>	CO/CO <sub>2</sub> (4/1) H <sub>2</sub> /CO <sub>2</sub> (4/1)	T: 60 °C pH: 6.0 GFL: 1 L/min LFR: 0.5 mL/min	BCR (1 L)	Acetate (25.0 g/L)	[51]
Stage 2: Lipids production	<i>Y. lipolytica</i>	Acetic acid	T: 35 °C pH: 7.3 DO: 20%	CSTR (2 L)	Lipids (18.0 g/L)	
• Syngas into Polyhydroxyalkanoates (PHAs)						
Stage 1: Syngas fermentation	<i>C. autoethanogenum</i>	30% CO, 10% CO <sub>2</sub> , 20% H <sub>2</sub> , 40% N <sub>2</sub>	T: 30 °C pH: 5.75 GFR: 0.01 L/min Agitation speed: 250 rpm	CSTR (2 L)	Acetate (2.7 g/L) Ethanol (3.8 g/L) 2,3-Butanediol (1.6 g/L)	[173]
Stage 2: PHA production	Mixed microbial culture (MMC)	Acetic acid	T: 30 °C	Glass bioreactor (1 L)	PHA (24% of cell dry weight)	
• Syngas into PHAs						
Stage 1: Syngas fermentation	<i>A. woodii</i>	20–65% CO, 2–14% H <sub>2</sub> , 16–42% CO <sub>2</sub> , 18–56% N <sub>2</sub>	N.A.	CSTR	Formate (1.89–2.79 g/L)	[176]
Stage 2: PHA production	<i>M. extorquens</i>	Formate	N.A.	CSTR (1 L)	Polyhydroxybutyrate (PHB) (0.097 g/L)	

GFR: gas flow rate; LFR: liquid flow rate; DO: dissolved oxygen.

### 5.3. Non-acetogenic Microorganisms as a Valid Alternative to Acetogens

Non-acetogenic microorganisms would represent a great opportunity for biotechnological applications of syngas fermentation, thereby producing high-value chemicals beyond acetate and ethanol. Hydrogenogenic strains are able to use CO as a carbon source via the biological water–gas shift (WGS) reaction (Figure 4). Several strains have been identified as able to conduct the biological WGS reaction, both mesophilic and thermophilic species, such as *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, *Citrobacter* sp. Y19, *Rubrivivax gelatinosus*, *Carboxydotherrmus hydrogenoformans*, and *Bacillus simithii* [20,177]. In particular, *R. rubrum* is the most studied strain due to its ability to produce H<sub>2</sub> during syngas fermentation [178]. *R. rubrum* is a purple non-sulfur bacterium that grows heterotrophically or autotrophically, using light as a source of energy via the Calvin–Benson–Bassham cycle in aerobic or anaerobic conditions, as well as in darkness. When CO is used as the sole carbon and energy source, it induces the expression of CODH and the CO-sensitive hydrogenase [179]. These enzymes act in the WGS reaction. The CO<sub>2</sub> released from this reaction is partly assimilated for cell material production, while the remaining CO<sub>2</sub> and the produced H<sub>2</sub> are released in the medium. Thus, the growth medium is enriched with H<sub>2</sub> during syngas fermentation [68]. Moreover, PHA production, especially in the form of PHB and polyhydroxyvalerate (PHV), from syngas fermentation with *R. rubrum* was demonstrated using 3-ketothiolase, acetoacetyl-CoA reductase, and PHB synthase [68,180]. Several research studies have been carried out to investigate the effect of light intensity, organic carbon source (i.e., acetate), and nutrient-limited media cultivation on microbial cell growth, metabolism, and H<sub>2</sub> and/or PHA production. These works showed that both energy sources of light [181] and organic substrates [182,183] are required to provide electrons to produce H<sub>2</sub>. Instead, nitrogen- [184] and/or phosphate-limiting [185] conditions are suitable for PHA accumulation in the cells.

Syngas fermentation with *R. rubrum* was also demonstrated in a 2 L CSTR for biological hydrogen production. The CSTR was operated with syngas (55% CO, 20% H<sub>2</sub>, 15% Ar, 10% CO<sub>2</sub>) under anaerobic conditions at various agitation speeds (150–500 rpm) and gas flow rates (5–14 mL/min), while the pH and temperature were set at constant values of 6.5 and 30 °C, respectively. Moreover, the fresh medium, which was fed at a 0.65 mL/min liquid flow rate, contained acetate as the organic carbon source. The H<sub>2</sub> productivity and yield were higher at the highest values of the agitation and gas flow rate [186,187].

The growth of *R. rubrum* was investigated using ground seed corn syngas and fermentation was initiated with an artificial syngas mixture (56.0% N<sub>2</sub>, 17.2% CO, 16.3% CO<sub>2</sub>, and 8.8% H<sub>2</sub>) that was used only to reach the desired density. In the batch fermentations, 340 mg/g cell protein/day of PHA was produced and was composed of 86% β-hydroxybutyrate and 14% β-hydroxyvalerate [180].

Hydrogen-oxidizing bacteria, so-called Knallgas bacteria, are able to produce PHA during aerobic syngas fermentation. These microorganisms are able to fix CO<sub>2</sub> into cell material using H<sub>2</sub> as the electron donor and O<sub>2</sub> as the electron acceptor. Among them, the most known and studied is *Ralstonia eutropha*. The fermentation technology for this microorganism was investigated to solve two major problems: (i) the gas mixture required for cell growth has a ratio of H<sub>2</sub>:CO<sub>2</sub>:O<sub>2</sub> equal to 7:1:1, which is in the explosive range and (ii) H<sub>2</sub> and O<sub>2</sub> show a low solubility in a liquid medium.

An explosion-proof fermentation bench-plant for aerobic gas fermentation with *R. eutropha* was developed that contained an oxygen concentration in the gas phase below 6.0%. The results showed cell and PHB productivities of 2.28 g/L/h and 1.55 g/L/h, respectively [188]. An approach similar to two-stage syngas fermentation was also employed to grow bacterial biomass heterotrophically and in an inorganic medium (H<sub>2</sub>, CO<sub>2</sub>) with a safe O<sub>2</sub> concentration (below 6.9%), followed by PHB accumulation up to 82.1% (*w/w*). In addition to two-stage fermentation in batch mode, the continuous production of PHB by two-stage fermentation was also investigated. In the first stage, fructose was continuously fed at a dilution rate of 0.1 h<sup>-1</sup>; then, the culture was transferred into an airlift fermenter in chemostatic conditions, obtaining an autotrophic PHB accumulation. The PHB productivity (0.025 g/L/h) and final concentration (57.6% *w/w*) were lower than for the batch fermentation, probably due to the limited availability of O<sub>2</sub> in the medium [189,190]. Moreover, after the addition into the culture medium of 0.05%

(*w/w*) carboxymethylcellulose (CMC) as surface-active reagent, the cellular content and productivity of PHB increased to 81.4% (*w/w*) and 1.02 g/L/h, respectively [191]. As a CO-resistant strain, *R. eutropha* was investigated for its PHA production with a gas mixture containing 5–25% (*v/v*) CO, which resulted in 70–75% PHA accumulation [192]. Although CO did not show any negative effects on the key enzymes of PHAs synthesis, it was not used as a co-substrate. To enable the use of CO, *R. eutropha* was engineered with CODH from *Oligotropha carboxidovorans* using a syngas mixture (40% CO, 40% H<sub>2</sub>, 10% CO<sub>2</sub>, and 10% N<sub>2</sub>) resulting in more than 20% PHB synthesis [193].

Finally, aerobic carboxydrotrophs are microorganisms known for using the reducing power of CO and H<sub>2</sub> to fix carbon through the Calvin cycle, with O<sub>2</sub> as the electron final acceptor. *O. carboxidovorans* is the most investigated carboxydrotroph. This microorganism grows in a CO, CO<sub>2</sub>, and H<sub>2</sub> mixture, and on organic substrates [194,195]. Interestingly, the chemolithoautotrophic growth of *O. carboxidovorans* in a gas mixture containing 50% air and 50% syngas (3% CH<sub>4</sub>, 18% CO<sub>2</sub>, 41% CO, and 38% H<sub>2</sub>) showed a modified viscosity of membrane lipids with longer chain fatty acids.

SYNPOL (“Biopolymers from syngas fermentation”), which was a European project that ended in 2016, aimed to improve acetogenic and non-acetogenic microorganisms’ performance during syngas fermentation to produce biopolymers. Several efforts were made to analyze the capacity of various bacteria to ferment syngas and to create genetically modified strains. Studies were carried out mostly on *R. rubrum*, *R. eutropha*, and *O. carboxidovorans* [196].

## 6. Summary and Outlook

Biomass gasification integrated with syngas fermentation is a promising model of second-generation biorefining. In the first step, gasification can be fed with different kinds of lignocellulosic biomass, from woody biomass to agro-industrial residues and herbaceous crops, thereby showing tremendous feedstock flexibility. In the second step, syngas fermentation can be performed by several microorganisms for the production of fuels and chemicals. Acetogens are the most widely used due to the Wood–Ljungdahl metabolic pathway, which allows them to produce acetate and ethanol as the main end products, but also other products, such as butyrate, butanol, caproate, hexanol, 2,3-butanediol, and lactate. Moreover, a mixed culture can also be used in a syngas fermentation process to produce alcohols, from ethanol to propanol, butanol, and hexanol, but they could contain human pathogens, i.e., *C. difficile* and *C. sordellii*. Over the past few years, considerable research has been done in the area of the design and optimization of syngas fermentation processes in terms of microbial nutritional requirements (i.e., organic carbon and nitrogen source, vitamins, trace metals, and reducing agent) and operating conditions (i.e., temperature, pH, and gaseous substrate partial pressure). The ability to control the acetogens’ metabolism through these parameters is important for improving the yields and productivity of the desired end product. The duration of the syngas fermentation processes reported in this review ranged from approximately 2 days (50 h) to 133 days (3192 h), with an average time of 35.5 days (852 h). The best fermentation performances were observed for *C. ragsdalei*, with a production of 25.26 g/L ethanol and an ethanol/acetate ratio of 5.24 in a 100 L CSTR during a 59-day process [140]. *C. carboxidovorans* showed production of 23.93 g/L of ethanol, an ethanol/acetate ratio of 6.96, and a productivity of 3.44 g/L/day in an 8 L HFMBR during a 19-day process [1]. *A. woodii* showed the highest acetate production (17.6 g/L) without byproduct formation in a CSTR with a submerged HFM module during an 8-day process [152]. *C. ljungdahlii* produced 14.97 g/L of acetate and 3.75 g/L of ethanol in a 3 L CSTR during a 45-day process [135]. *C. ragsdalei* produced 12.30 g/L of acetate and 5.7 g/L of ethanol in a 1 L TBR in approximately 70 days [144]. Alternative reactor configurations should be considered for the syngas fermentation process, such as a bubble column reactor, trickle-bed reactor, membrane bioreactor, and monolithic biofilm reactor. In recent years, research activity has intensified in the field of biofilm reactors to achieve higher cell density than in suspended growth reactors. Among them, the hollow-fiber membrane bioreactor is the most promising configuration due to its high mass transfer rates and low energy consumption. The major challenge is the selection of a suitable membrane material. The latter should overcome the gas–liquid mass transfer

limitation and improve biofilm formation. Moreover, the membrane material should display long-term stability during the process, avoiding pore-wetting, biofouling, and other related problems. Therefore, further research studies are essential for developing an optimal reactor configuration that allows for high process performance, scalability, and implementation at an industrial scale. On the other hand, the development of genetically modified strains that are able to perform syngas fermentation represents a valid approach to improving process efficiency. Recent advances in the synthetic biology and metabolic engineering of acetogens are driven by two major objectives: to increase the yield and productivity of native products obtained from the Wood–Ljungdahl pathway, mainly acetate and ethanol, and to expand the products spectrum of acetogens, especially of *C. autoethanogenum* and *C. ljungdahlii*. In the latter case, the two strains were engineered to produce butanol, acetone, and isopropanol, along with other high-value compounds, such as 3-hydroxypropionate (3-HP), 2-butanol, methyl ethyl ketone (MEK), biodiesel, polyhydroxybutyrate (PHB), and terpenes. Therefore, genetic tools offer great opportunities to increase syngas fermentation valorization, although further efforts are required to provide a solid strain design strategy.

The potential of this second-generation biorefinery model is demonstrated by the large-scale projects of two companies in cooperation, LanzaTech Inc. and Aemetis, Inc. LanzaTech Inc. is mainly working in New Zealand, China, and Taiwan using steel mill off-gases for the production of ethanol through syngas fermentation with a proprietary microorganism. Aemetis, Inc., instead, developed a full-scale operating biorefinery for ethanol production using syngas derived from waste biomass gasification (i.e., waste orchard wood and almond and walnut shells).

In the future, additional approaches need to be developed to improve the potentiality and sustainability of this integrated biorefinery model, such as the development of efficient and affordable syngas clean-up strategies, the coupling of syngas fermentation with additional bioprocesses to produce high-value chemicals, and the investigation of promising non-acetogenic microorganisms to improve product formation and reduce the production cost.

**Author Contributions:** Conceptualization, C.C., A.B., and I.P.; methodology, C.C., A.B., and I.P.; resources, C.C., A.B., and I.P.; writing—original draft preparation, C.C., A.B., and I.P.; writing—review and editing, C.C., A.B., G.A., I.d.B., R.A., G.B., and I.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** The authors gratefully acknowledge the financial support from the Interuniversity Consortium for Biotechnology (CIB), Italy.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Cherubini, F. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. *Energy Convers. Manag.* **2010**, *51*, 1412–1421. [[CrossRef](#)]
2. Halkos, G.E.; Gkampoura, E.C. Reviewing usage, potentials, and limitations of renewable energy sources. *Energies* **2020**, *13*, 2906. [[CrossRef](#)]
3. Michaelides, E.E. *Alternative Energy Sources*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2012.
4. The European Commission. *The European Green Deal*; The European Commission: Brussels, Belgium, 2019.
5. Org, S.U. *Transforming Our World: The 2030 Agenda for Sustainable Development*; United Nations: New York, NY, USA, 2015.
6. Clark, J.H.; Deswarte, F.E.I. *Introduction to Chemicals from Biomass*; John Wiley & Sons: Hoboken, NJ, USA, 2014; ISBN 9781118714461.
7. Panwar, N.L.; Kaushik, S.C.; Kothari, S. Role of renewable energy sources in environmental protection: A review. *Renew. Sustain. Energy Rev.* **2011**, *15*, 1513–1524. [[CrossRef](#)]

8. European Commission. *Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC (Text with EEA Relevance)*; Council of the European Community: Brussels, Belgium, 2009.
9. Dubois, O.; Gomez San Juan, M. How sustainability is addressed in official bioeconomy strategies at international, national, and regional levels—An overview. *Environ. Nat. Resour. Manag. Work. Pap. (FAO)* **2016**, *63*, 1–33.
10. Aristizábal-Marulanda, V.; Cardona Alzate, C.A. Methods for designing and assessing biorefineries: Review. *Biofuels Bioprod. Biorefining* **2019**, *13*, 789–808. [[CrossRef](#)]
11. Cherubini, F.; Jungmeier, G.; Wellisch, M.; Willke, T.; Skiadas, I.; van Ree, R.; de Jong, E. Toward a common classification approach for biorefinery systems. *Biofuels Bioprod. Biorefining* **2009**, *3*, 534–546. [[CrossRef](#)]
12. Nguyen, Q.; Bowyer, J.; Howe, J.; Bratkovich, S.; Groot, H.; Pepke, E.; Fernholz, K. *Global Production of Second Generation Biofuels: Trends and Influences*; Dovetail Partners Global Production of Second Generation Biofuels: Trends and Influences; Dovetail Partners Inc.: Minneapolis, MN, USA, 2017.
13. Saladini, F.; Patrizi, N.; Pulselli, F.M.; Marchettini, N.; Bastianoni, S. Guidelines for emergy evaluation of first, second and third generation biofuels. *Renew. Sustain. Energy Rev.* **2016**, *66*, 221–227. [[CrossRef](#)]
14. 3. Production and Uses of Aquatic Biomass in: Biorefineries. Available online: <https://www.degruyter.com/view/book/9783110331585/10.1515/9783110331585-007.xml> (accessed on 30 October 2020).
15. Leong, W.H.; Lim, J.W.; Lam, M.K.; Uemura, Y.; Ho, Y.C. Third generation biofuels: A nutritional perspective in enhancing microbial lipid production. *Renew. Sustain. Energy Rev.* **2018**, *91*, 950–961. [[CrossRef](#)]
16. Dahmen, N.; Lewandowski, I.; Zibek, S.; Weidtmann, A. Integrated lignocellulosic value chains in a growing bioeconomy: Status quo and perspectives. *GCB Bioenergy* **2019**, *11*, 107–117. [[CrossRef](#)]
17. Isikgor, F.H.; Becer, C.R. Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers. *Polym. Chem.* **2015**, *6*, 4497–4559. [[CrossRef](#)]
18. Sansaniwal, S.K.; Rosen, M.A.; Tyagi, S.K. Global challenges in the sustainable development of biomass gasification: An overview. *Renew. Sustain. Energy Rev.* **2017**, *80*, 23–43. [[CrossRef](#)]
19. Acharya, B.; Roy, P.; Dutta, A. Review of syngas fermentation processes for bioethanol. *Biofuels* **2014**, *5*, 551–564. [[CrossRef](#)]
20. Mohammadi, M.; Najafpour, G.D.; Younesi, H.; Lahijani, P.; Uzir, M.H.; Mohamed, A.R. Bioconversion of synthesis gas to second generation biofuels: A review. *Renew. Sustain. Energy Rev.* **2011**, *15*, 4255–4273. [[CrossRef](#)]
21. Munasinghe, P.C.; Khanal, S.K. Biomass-derived syngas fermentation into biofuels: Opportunities and challenges. *Bioresour. Technol.* **2010**, *101*, 5013–5022. [[CrossRef](#)]
22. Molino, A.; Chianese, S.; Musmarra, D. Biomass gasification technology: The state of the art overview. *J. Energy Chem.* **2016**, *25*, 10–25. [[CrossRef](#)]
23. Rauch, R.; Hrbek, J.; Hofbauer, H. Biomass gasification for synthesis gas production and applications of the syngas. *Wiley Interdiscip. Rev. Energy Environ.* **2014**, *3*, 343–362. [[CrossRef](#)]
24. Devarapalli, M.; Atiyeh, H.K. A review of conversion processes for bioethanol production with a focus on syngas fermentation. *Biofuel Res. J.* **2015**, *2*, 268–280. [[CrossRef](#)]
25. Neubauer, Y.; Liu, H. Biomass gasification. *Biomass Combust. Sci. Technol. Eng.* **2013**, *8*, 106–129.
26. Sikarwar, V.S.; Zhao, M.; Clough, P.; Yao, J.; Zhong, X.; Memon, M.Z.; Shah, N.; Anthony, E.J.; Fennell, P.S. An overview of advances in biomass gasification. *Energy Environ. Sci.* **2016**, *9*, 2939–2977. [[CrossRef](#)]
27. Sansaniwal, S.K.; Pal, K.; Rosen, M.A.; Tyagi, S.K. Recent advances in the development of biomass gasification technology: A comprehensive review. *Renew. Sustain. Energy Rev.* **2017**, *72*, 363–384. [[CrossRef](#)]
28. Dai, J.; Saayman, J.; Grace, J.R.; Ellis, N. Gasification of Woody Biomass. *Annu. Rev. Chem. Biomol. Eng.* **2015**, *6*, 77–99. [[CrossRef](#)] [[PubMed](#)]
29. Kennes, D.; Abubackar, H.N.; Diaz, M.; Veiga, M.C.; Kennes, C. Bioethanol production from biomass: Carbohydrate vs syngas fermentation. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 304–317. [[CrossRef](#)]
30. IEA BioEnergy Agreement Task 33: Thermal Gasification of Biomass. Available online: <http://task33.ieabioenergy.com/> (accessed on 1 November 2020).
31. Xu, D.; Tree, D.R.; Lewis, R.S. The effects of syngas impurities on syngas fermentation to liquid fuels. *Biomass Bioenergy* **2011**, *35*, 2690–2696. [[CrossRef](#)]

32. Ciferno, J.P.; Marano, J.J. *Benchmarking Biomass Gasification Technologies for Fuels, Chemicals and Hydrogen Production*; US DOE National Energy Technology Laboratory: Pittsburgh, PA, USA, 2002.
33. Kruse, A. Supercritical water gasification. *Biofuels Bioprod. Biorefining* **2008**, *2*, 415–437. [[CrossRef](#)]
34. Yang, H.; Chen, H. *Biomass Gasification for Synthetic Liquid Fuel Production*; All rights reserved; Woodhead Publishing Limited: Sawston, UK, 2015; ISBN 9780857098085.
35. Cheng, Y.; Thow, Z.; Wang, C.H. Biomass gasification with CO<sub>2</sub> in a fluidized bed. *Powder Technol.* **2016**, *296*, 87–101. [[CrossRef](#)]
36. Ren, J.; Cao, J.P.; Zhao, X.Y.; Yang, F.L.; Wei, X.Y. Recent advances in syngas production from biomass catalytic gasification: A critical review on reactors, catalysts, catalytic mechanisms and mathematical models. *Renew. Sustain. Energy Rev.* **2019**, *116*, 109426. [[CrossRef](#)]
37. Ramachandriya, K.D.; Kundiyana, D.K.; Sharma, A.M.; Kumar, A.; Atiyeh, H.K.; Huhnke, R.L.; Wilkins, M.R. Critical factors affecting the integration of biomass gasification and syngas fermentation technology. *AIMS Bioeng.* **2016**, *3*, 188–210. [[CrossRef](#)]
38. Chen, W.; Annamalai, K.; Ansley, R.J.; Mirik, M. Updraft fixed bed gasification of mesquite and juniper wood samples. *Energy* **2012**, *41*, 454–461. [[CrossRef](#)]
39. Huang, F.; Jin, S. Investigation of biomass (pine wood) gasification: Experiments and Aspen Plus simulation. *Energy Sci. Eng.* **2019**, *7*, 1178–1187. [[CrossRef](#)]
40. Yan, Q.; Yu, F.; Liu, J.; Street, J.; Gao, J.; Cai, Z.; Zhang, J. Catalytic conversion wood syngas to synthetic aviation turbine fuels over a multifunctional catalyst. *Bioresour. Technol.* **2013**, *127*, 281–290. [[CrossRef](#)]
41. Galvagno, S.; Casciaro, G.; Casu, S.; Martino, M.; Mingazzini, C.; Russo, A.; Portofino, S. Steam gasification of tyre waste, poplar, and refuse-derived fuel: A comparative analysis. *Waste Manag.* **2009**, *29*, 678–689. [[CrossRef](#)] [[PubMed](#)]
42. de Oliveira, J.L.; da Silva, J.N.; Martins, M.A.; Pereira, E.G. Gasification of waste from coffee and eucalyptus production as an alternative source of bioenergy in Brazil. *Sustain. Energy Technol. Assess.* **2018**, *27*, 159–166. [[CrossRef](#)]
43. Kaewluan, S.; Pipatmanomai, S. Potential of synthesis gas production from rubber wood chip gasification in a bubbling fluidised bed gasifier. In *Energy Conversion and Management*; Elsevier Ltd.: Amsterdam, The Netherlands, 2011; Volume 52, pp. 75–84.
44. Nipattummakul, N.; Ahmed, I.I.; Kerdsuwan, S.; Gupta, A.K. Steam gasification of oil palm trunk waste for clean syngas production. *Appl. Energy* **2012**, *92*, 778–782. [[CrossRef](#)]
45. Mikeska, M.; Najser, J.; Peer, V.; Frantik, J.; Kielar, J. Quality assessment of gas produced from different types of biomass pellets in gasification process. *Energy Explor. Exploit.* **2020**, *38*, 406–416. [[CrossRef](#)]
46. Peng, W.X.; Wang, L.S.; Mirzaee, M.; Ahmadi, H.; Esfahani, M.J.; Fremaux, S. Hydrogen and syngas production by catalytic biomass gasification. *Energy Convers. Manag.* **2017**, *135*, 270–273. [[CrossRef](#)]
47. Carpenter, D.L.; Bain, R.L.; Davis, R.E.; Dutta, A.; Feik, C.J.; Gaston, K.R.; Jablonski, W.; Phillips, S.D.; Nimlos, M.R. Pilot-scale gasification of corn stover, switchgrass, wheat straw, and wood: 1. Parametric study and comparison with literature. *Ind. Eng. Chem. Res.* **2010**, *49*, 1859–1871. [[CrossRef](#)]
48. Weiland, F.; Nordwaeger, M.; Olofsson, I.; Wiinikka, H.; Nordin, A. Entrained flow gasification of torrefied wood residues. *Fuel Process. Technol.* **2014**, *125*, 51–58. [[CrossRef](#)]
49. Vonk, G.; Piriou, B.; Dos Santos, P.F.; Wolbert, D.; Väitilingom, G. Comparative analysis of wood and solid recovered fuels gasification in a downdraft fixed bed reactor. *Waste Manag.* **2019**, *85*, 106–120. [[CrossRef](#)]
50. Arena, U.; Zaccariello, L.; Mastellone, M.L. Gasification of Natural and Waste Biomass in a Pilot Scale Fluidized Bed Reactor. *Combust. Sci. Technol.* **2010**, *182*, 625–639. [[CrossRef](#)]
51. Hu, P.; Chakraborty, S.; Kumar, A.; Woolston, B.; Liu, H.; Emerson, D.; Stephanopoulos, G. Integrated bioprocess for conversion of gaseous substrates to liquids. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3773–3778. [[CrossRef](#)]
52. Wu, C.Z.; Yin, X.L.; Ma, L.L.; Zhou, Z.Q.; Chen, H.P. Operational characteristics of a 1.2-MW biomass gasification and power generation plant. *Biotechnol. Adv.* **2009**, *27*, 588–592. [[CrossRef](#)] [[PubMed](#)]
53. Senapati, P.K.; Behera, S. Experimental investigation on an entrained flow type biomass gasification system using coconut coir dust as powdery biomass feedstock. *Bioresour. Technol.* **2012**, *117*, 99–106. [[CrossRef](#)] [[PubMed](#)]

54. Singh, D.; Yadav, S.; Rajesh, V.M.; Mohanty, P. Groundnut shell gasification performance in a fluidized bed gasifier with bubbling air as gasification medium. *Environ. Technol.* **2019**, *40*, 3140–3152. [[CrossRef](#)] [[PubMed](#)]
55. Rapagnà, S. Steam-gasification of biomass in a fluidised-bed of olivine particles. *Biomass Bioenergy* **2000**, *19*, 187–197. [[CrossRef](#)]
56. Dogru, M.; Howarth, C.R.; Akay, G.; Keskinler, B.; Malik, A.A. Gasification of hazelnut shells in a downdraft gasifier. *Energy* **2002**, *27*, 415–427. [[CrossRef](#)]
57. García-Ibañez, P.; Cabanillas, A.; Sánchez, J.M. Gasification of leached orujillo (olive oil waste) in a pilot plant circulating fluidised bed reactor. Preliminary results. *Biomass Bioenergy* **2004**, *27*, 183–194. [[CrossRef](#)]
58. Biagini, E.; Barontini, F.; Tognotti, L. Gasification of agricultural residues in a demonstrative plant: Corn cobs. *Bioresour. Technol.* **2015**, *173*, 110–116. [[CrossRef](#)]
59. Biagini, E.; Barontini, F.; Tognotti, L. Gasification of Agricultural Residues in a Demonstrative Plant. *Bioresour. Technol.* **2014**, *37*, 110–116. [[CrossRef](#)]
60. Chiodo, V.; Urbani, F.; Zafarana, G.; Prestipino, M.; Galvagno, A.; Maisano, S. Syngas production by catalytic steam gasification of citrus residues. *Int. J. Hydrogen Energy* **2017**, *42*, 28048–28055. [[CrossRef](#)]
61. Maisano, S.; Urbani, F.; Cipiti, F.; Freni, F.; Chiodo, V. Syngas production by BFB gasification: Experimental comparison of different biomasses. *Int. J. Hydrogen Energy* **2019**, *44*, 4414–4422. [[CrossRef](#)]
62. Erlich, C.; Fransson, T.H. Downdraft gasification of pellets made of wood, palm-oil residues respective bagasse: Experimental study. *Appl. Energy* **2011**, *88*, 899–908. [[CrossRef](#)]
63. Gabra, M.; Pettersson, E.; Backman, R.; Kjellström, B. Evaluation of cyclone gasifier performance for gasification of sugar cane residue—Part 1: Gasification of bagasse. *Biomass Bioenergy* **2001**, *21*, 351–369. [[CrossRef](#)]
64. Werle, S. Impact of feedstock properties and operating conditions on sewage sludge gasification in a fixed bed gasifier. *Waste Manag. Res.* **2014**, *32*, 954–960. [[CrossRef](#)]
65. Xue, G.; Kwapinska, M.; Horvat, A.; Kwapinski, W.; Rabou, L.P.L.M.; Dooley, S.; Czajka, K.M.; Leahy, J.J. Gasification of torrefied *Miscanthus x giganteus* in an air-blown bubbling fluidized bed gasifier. *Bioresour. Technol.* **2014**, *159*, 397–403. [[CrossRef](#)] [[PubMed](#)]
66. Christodoulou, C.; Tsekos, C.; Tsalidis, G.; Fantini, M.; Panopoulos, K.D.; De Jong, W.; Kakaras, E. Attempts on cardoon gasification in two different circulating fluidized beds. *Case Stud. Therm. Eng.* **2014**, *4*, 42–52. [[CrossRef](#)]
67. Torreiro, Y.; Ortiz, I.; Molina, G.; Maroño, M.; Pérez, V.; Murillo, J.M.; Ramos, R.; Fernández, M.; García, S.; Sánchez, J.M. Thermochemical assessment of *Nicotiana glauca*, *Panicum virgatum* and *Elytrigia elongata* as fuels for energy recovery through gasification. *Fuel* **2018**, *225*, 71–79. [[CrossRef](#)]
68. Drzyzga, O.; Revelles, O.; Durante-Rodríguez, G.; Díaz, E.; García, J.L.; Prieto, A. New challenges for syngas fermentation: Towards production of biopolymers. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 1735–1751. [[CrossRef](#)]
69. Drake, H.L.; Gößner, A.S.; Daniel, S.L. Old acetogens, new light. *Ann. N. Y. Acad. Sci.* **2008**, *1125*, 100–128. [[CrossRef](#)]
70. Min, F.; Kopke, M.; Dennis, S. Gas Fermentation for Commercial Biofuels Production. *Liq. Gaseous Solid Biofuels Convers. Tech.* **2013**, 125–173. [[CrossRef](#)]
71. Phillips, J.R.; Huhnke, R.L.; Atiyeh, H.K. Syngas fermentation: A microbial conversion process of gaseous substrates to various products. *Fermentation* **2017**, *3*, 28. [[CrossRef](#)]
72. Sun, X.; Atiyeh, H.K.; Huhnke, R.L.; Tanner, R.S. Syngas fermentation process development for production of biofuels and chemicals: A review. *Bioresour. Technol. Rep.* **2019**, *7*, 100279. [[CrossRef](#)]
73. Bertsch, J.; Müller, V. Bioenergetic constraints for conversion of syngas to biofuels in acetogenic bacteria. *Biotechnol. Biofuels* **2015**, *8*, 1–12. [[CrossRef](#)] [[PubMed](#)]
74. Müller, V. Energy Conservation in Acetogenic Bacteria. *Appl. Environ. Microbiol.* **2003**, *69*, 6345–6353. [[CrossRef](#)]
75. Koepke, M.; Liew, F. Recombinant microorganism and methods of production thereof. U.S. Patent Application No. 2011/0236941, 29 September 2011.
76. Bengelsdorf, F.R.; Straub, M.; Dürre, P. Bacterial synthesis gas (syngas) fermentation. *Environ. Technol.* **2013**, *34*, 1639–1651. [[CrossRef](#)]
77. Schuchmann, K.; Müller, V. Autotrophy at the thermodynamic limit of life: A model for energy conservation in acetogenic bacteria. *Nat. Rev. Microbiol.* **2014**, *12*, 809–821. [[CrossRef](#)]



78. Balch, W.E.; Schoberth, S.; Tanner, R.S.; Wolfe, R.S. *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. *Int. J. Syst. Bacteriol.* **1977**, *27*, 355–361. [[CrossRef](#)]
79. Wieringa, K.T. The formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. *Antonie Van Leeuwenhoek* **1939**, *6*, 251–262. [[CrossRef](#)]
80. Abrini, J.; Naveau, H.; Nyns, E. *Clostridium autoethanogenum*, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. *Arch. Microbiol.* **1994**, *161*, 345–351. [[CrossRef](#)]
81. Liou, J.S.C.; Balkwill, D.L.; Drake, G.R.; Tanner, R.S. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 2085–2091. [[CrossRef](#)]
82. Huhnke, R.L.; Lewis, R.S.; Tanner, R.S. Isolation and Characterization of Novel Clostridial Species. U.S. Patent 7,704,723, 27 April 2010.
83. Tanner, R.S.; Miller, L.M.; Yang, D. Clostridial rRNA Homology Group I. *Int. J. Syst. Bacteriol.* **1993**, *43*, 232–236. [[CrossRef](#)]
84. Ragsdale, S.W.; Pierce, E. Acetogenesis and the Wood-Ljungdahl pathway of CO<sub>2</sub> fixation. *Biochim. Biophys. Acta Proteins Proteom.* **2008**, *1784*, 1873–1898. [[CrossRef](#)] [[PubMed](#)]
85. Heiskanen, H.; Virkajärvi, I.; Viikari, L. The effect of syngas composition on the growth and product formation of *Butyrivibrio methylotrophicus*. *Enzyme Microb. Technol.* **2007**, *41*, 362–367. [[CrossRef](#)]
86. Gößner, A.S.; Picardal, F.; Tanner, R.S.; Drake, H.L. Carbon metabolism of the moderately acid-tolerant acetogen *Clostridium drakei* isolated from peat. *FEMS Microbiol. Lett.* **2008**, *287*, 236–242. [[PubMed](#)]
87. Chang, I.S.; Kim, B.H.; Lovitt, R.W.; Bang, J.S. Effect of CO partial pressure on cell-recycled continuous CO fermentation by *Eubacterium limosum* KIST612. *Process Biochem.* **2001**, *37*, 411–421. [[CrossRef](#)]
88. Xu, S.; Fu, B.; Zhang, L.; Liu, H. Bioconversion of H<sub>2</sub>/CO<sub>2</sub> by acetogen enriched cultures for acetate and ethanol production: The impact of pH. *World J. Microbiol. Biotechnol.* **2015**, *31*, 941–950. [[CrossRef](#)]
89. Chakraborty, S.; Rene, E.R.; Lens, P.N.L.; Veiga, M.C.; Kennes, C. Enrichment of a solventogenic anaerobic sludge converting carbon monoxide and syngas into acids and alcohols. *Bioresour. Technol.* **2019**, *272*, 130–136. [[CrossRef](#)]
90. Alves, J.I.; Stams, A.J.M.; Plugge, C.M.; Madalena Alves, M.; Sousa, D.Z. Enrichment of anaerobic syngas-converting bacteria from thermophilic bioreactor sludge. *FEMS Microbiol. Ecol.* **2013**, *86*, 590–597. [[CrossRef](#)]
91. Park, S.; Yasin, M.; Kim, D.; Park, H.D.; Kang, C.M.; Kim, D.J.; Chang, I.S. Rapid enrichment of (homo)acetogenic consortia from animal feces using a high mass-transfer gas-lift reactor fed with syngas. *J. Ind. Microbiol. Biotechnol.* **2013**, *40*, 995–1003. [[CrossRef](#)]
92. Singla, A.; Verma, D.; Lal, B.; Sarma, P.M. Enrichment and optimization of anaerobic bacterial mixed culture for conversion of syngas to ethanol. *Bioresour. Technol.* **2014**, *172*, 41–49. [[CrossRef](#)]
93. Buschhorn, H.; Durre, P.; Gottschalk, G. Production and utilization of ethanol by the homoacetogen *Acetobacterium woodii*. *Appl. Environ. Microbiol.* **1989**, *55*, 1835–1840. [[CrossRef](#)]
94. Braun, M.; Mayer, F.; Gottschalk, G. *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Arch. Microbiol.* **1981**, *128*, 288–293. [[CrossRef](#)] [[PubMed](#)]
95. Phillips, J.R.; Atiyeh, H.K.; Tanner, R.S.; Torres, J.R.; Saxena, J.; Wilkins, M.R.; Huhnke, R.L. Butanol and hexanol production in *Clostridium carboxidivorans* syngas fermentation: Medium development and culture techniques. *Bioresour. Technol.* **2015**, *190*, 114–121. [[CrossRef](#)] [[PubMed](#)]
96. Ramió-Pujol, S.; Ganigué, R.; Bañeras, L.; Colprim, J. Incubation at 25 °C prevents acid crash and enhances alcohol production in *Clostridium carboxidivorans* P7. *Bioresour. Technol.* **2015**, *192*, 296–303. [[CrossRef](#)] [[PubMed](#)]
97. Richter, H.; Molitor, B.; Wei, H.; Chen, W.; Aristilde, L.; Angenent, L.T. Ethanol production in syngas-fermenting: *Clostridium ljungdahlii* is controlled by thermodynamics rather than by enzyme expression. *Energy Environ. Sci.* **2016**, *9*, 2392–2399. [[CrossRef](#)]
98. Allen, T.D.; Caldwell, M.E.; Lawson, P.A.; Huhnke, R.L.; Tanner, R.S. *Alkalibaculum bacchi* gen. nov., sp. nov., a CO-oxidizing, ethanol-producing acetogen isolated from livestock-impacted soil. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2483–2489. [[CrossRef](#)] [[PubMed](#)]
99. Cardon, B.P.; Barker, H.A. Two New Amino-Acid-Fermenting Bacteria, *Clostridium propionicum* and *Diplococcus glycinophilus*. *J. Bacteriol.* **1946**, *52*, 629–634. [[CrossRef](#)]

100. Liu, K.; Atiyeh, H.K.; Stevenson, B.S.; Tanner, R.S.; Wilkins, M.R.; Huhnke, R.L. Mixed culture syngas fermentation and conversion of carboxylic acids into alcohols. *Bioresour. Technol.* **2014**, *152*, 337–346. [[CrossRef](#)]
101. Diender, M.; Stams, A.J.M.; Sousa, D.Z. Production of medium-chain fatty acids and higher alcohols by a synthetic co-culture grown on carbon monoxide or syngas. *Biotechnol. Biofuels* **2016**, *9*, 1–11. [[CrossRef](#)]
102. Richter, H.; Molitor, B.; Diender, M.; Sousa, D.Z.; Angenent, L.T. A narrow pH range supports butanol, hexanol, and octanol production from syngas in a continuous co-culture of *Clostridium ljungdahlii* and *Clostridium kluyveri* with in-line product extraction. *Front. Microbiol.* **2016**, *7*, 1773. [[CrossRef](#)]
103. Liew, F.; Koepke, M. Recombinant microorganisms make biodiesel. U.S. Patent 9,347,076, 21 June 2013.
104. Liew, F.M.; Martin, M.E.; Tappel, R.C.; Heijstra, B.D.; Mihalcea, C.; Köpke, M. Gas Fermentation—A flexible platform for commercial scale production of low-carbon-fuels and chemicals from waste and renewable feedstocks. *Front. Microbiol.* **2016**, *7*, 694. [[CrossRef](#)]
105. Abubackar, H.N.; Veiga, M.C.; Kennes, C. Biological conversion of carbon monoxide to ethanol: Effect of pH, gas pressure, reducing agent and yeast extract. *Bioresour. Technol.* **2012**, *114*, 518–522. [[CrossRef](#)] [[PubMed](#)]
106. Park, S.; Ahn, B.; Kim, Y.K. Growth enhancement of bioethanol-producing microbe *Clostridium autoethanogenum* by changing culture medium composition. *Bioresour. Technol. Reports* **2019**, *6*, 237–240. [[CrossRef](#)]
107. Barik, S.; Prieto, S.; Harrison, S.B.; Clausen, E.C.; Gaddy, J.L. Biological production of alcohols from coal through indirect liquefaction - Scientific note. *Appl. Biochem. Biotechnol.* **1988**, *18*, 363–378. [[CrossRef](#)]
108. Cotter, J.L.; Chinn, M.S.; Grunden, A.M. Ethanol and acetate production by *Clostridium ljungdahlii* and *Clostridium autoethanogenum* using resting cells. *Bioprocess Biosyst. Eng.* **2009**, *32*, 369–380. [[CrossRef](#)]
109. Abubackar, H.N.; Veiga, M.C.; Kennes, C. Carbon monoxide fermentation to ethanol by *Clostridium autoethanogenum* in a bioreactor with no accumulation of acetic acid. *Bioresour. Technol.* **2015**, *186*, 122–127. [[CrossRef](#)]
110. Saxena, J.; Tanner, R.S. Effect of trace metals on ethanol production from synthesis gas by the ethanologenic acetogen, *Clostridium ragsdalei*. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 513–521. [[CrossRef](#)]
111. Shen, Y.; Brown, R.C.; Wen, Z. Syngas fermentation by *Clostridium carboxidivorans* P7 in a horizontal rotating packed bed biofilm reactor with enhanced ethanol production. *Appl. Energy* **2017**, *187*, 585–594. [[CrossRef](#)]
112. Li, D.; Meng, C.; Wu, G.; Xie, B.; Han, Y.; Guo, Y.; Song, C.; Gao, Z.; Huang, Z. Effects of zinc on the production of alcohol by *Clostridium carboxidivorans* P7 using model syngas. *J. Ind. Microbiol. Biotechnol.* **2018**, *45*, 61–69. [[CrossRef](#)]
113. Sim, J.H.; Kamaruddin, A.H. Optimization of acetic acid production from synthesis gas by chemolithotrophic bacterium-*Clostridium acetium* using statistical approach. *Bioresour. Technol.* **2008**, *99*, 2724–2735. [[CrossRef](#)]
114. Abubackar, H.N.; Veiga, M.C.; Kennes, C. Biological conversion of carbon monoxide: Rich syngas or waste gases to bioethanol. *Biofuels Bioprod. Biorefining* **2011**, *5*, 93–114. [[CrossRef](#)]
115. Kundiyana, D.K.; Huhnke, R.L.; Maddipati, P.; Atiyeh, H.K.; Wilkins, M.R. Feasibility of incorporating cotton seed extract in *Clostridium* strain P11 fermentation medium during synthesis gas fermentation. *Bioresour. Technol.* **2010**, *101*, 9673–9680. [[CrossRef](#)] [[PubMed](#)]
116. Maddipati, P.; Atiyeh, H.K.; Bellmer, D.D.; Huhnke, R.L. Ethanol production from syngas by *Clostridium* strain P11 using corn steep liquor as a nutrient replacement to yeast extract. *Bioresour. Technol.* **2011**, *102*, 6494–6501. [[CrossRef](#)] [[PubMed](#)]
117. Saxena, J.; Tanner, R.S. Optimization of a corn steep medium for production of ethanol from synthesis gas fermentation by *Clostridium ragsdalei*. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1553–1561. [[CrossRef](#)]
118. Ramchandriya, K.D.; Kundiyana, D.K.; Wilkins, M.R.; Terrill, J.B.; Atiyeh, H.K.; Huhnke, R.L. Carbon dioxide conversion to fuels and chemicals using a hybrid green process. *Appl. Energy* **2013**, *112*, 289–299. [[CrossRef](#)]
119. Shen, S.; Gu, Y.; Chai, C.; Jiang, W.; Zhuang, Y.; Wang, Y. Enhanced alcohol titre and ratio in carbon monoxide-rich off-gas fermentation of *Clostridium carboxidivorans* through combination of trace metals optimization with variable-temperature cultivation. *Bioresour. Technol.* **2017**, *239*, 236–243. [[CrossRef](#)]
120. Fernández-Naveira, Á.; Abubackar, H.N.; Veiga, M.C.; Kennes, C. Efficient butanol-ethanol (B-E) production from carbon monoxide fermentation by *Clostridium carboxidivorans*. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 3361–3370. [[CrossRef](#)]

121. Arslan, K.; Bayar, B.; Nalakath Abubackar, H.; Veiga, M.C.; Kennes, C. Solventogenesis in *Clostridium acetivum* producing high concentrations of ethanol from syngas. *Bioresour. Technol.* **2019**, *292*. [CrossRef]
122. Abubackar, H.N.; Bengelsdorf, F.R.; Dürre, P.; Veiga, M.C.; Kennes, C. Improved operating strategy for continuous fermentation of carbon monoxide to fuel-ethanol by clostridia. *Appl. Energy* **2016**, *169*, 210–217. [CrossRef]
123. Martin, M.E.; Richter, H.; Saha, S.; Angenent, L.T. Traits of selected *Clostridium* strains for syngas fermentation to ethanol. *Biotechnol. Bioeng.* **2016**, *113*, 531–539. [CrossRef]
124. Abubackar, H.N.; Veiga, M.C.; Kennes, C. Production of acids and alcohols from syngas in a two-stage continuous fermentation process. *Bioresour. Technol.* **2018**, *253*, 227–234. [CrossRef]
125. Richter, H.; Martin, M.E.; Angenent, L.T. A two-stage continuous fermentation system for conversion of syngas into ethanol. *Energies* **2013**, *6*, 3987–4000. [CrossRef]
126. Hurst, K.M.; Lewis, R.S. Carbon monoxide partial pressure effects on the metabolic process of syngas fermentation. *Biochem. Eng. J.* **2010**, *48*, 159–165. [CrossRef]
127. Sim, J.H.; Kamaruddin, A.H.; Long, W.S.; Najafpour, G. *Clostridium acetivum*-A potential organism in catalyzing carbon monoxide to acetic acid: Application of response surface methodology. *Enzyme Microb. Technol.* **2007**, *40*, 1234–1243. [CrossRef]
128. Mayer, A.; Schädler, T.; Trunz, S.; Stelzer, T.; Weuster-Botz, D. Carbon monoxide conversion with *Clostridium acetivum*. *Biotechnol. Bioeng.* **2018**, *115*, 2740–2750. [CrossRef] [PubMed]
129. Demler, M.; Weuster-Botz, D. Reaction engineering analysis of hydrogenotrophic production of acetic acid by *Acetobacterium woodii*. *Biotechnol. Bioeng.* **2011**, *108*, 470–474. [CrossRef] [PubMed]
130. Najafpour, G.; Younesi, H. Ethanol and acetate synthesis from waste gas using batch culture of *Clostridium ljungdahlii*. *Enzyme Microb. Technol.* **2006**, *38*, 223–228. [CrossRef]
131. Klasson, K.T.; Ackerson, M.D.; Clausen, E.C.; Gaddy, J.L. Bioreactor design for synthesis gas fermentations. *Fuel* **1991**, *70*, 605–614. [CrossRef]
132. Mohammadi, M.; Younesi, H.; Najafpour, G.; Mohamed, A.R. Sustainable ethanol fermentation from synthesis gas by *Clostridium ljungdahlii* in a continuous stirred tank bioreactor. *J. Chem. Technol. Biotechnol.* **2012**, *87*, 837–843. [CrossRef]
133. Younesi, H.; Najafpour, G.; Mohamed, A.R. Liquid fuel production from synthesis gas via fermentation process in a continuous tank bioreactor (CSTBR) using *Clostridium ljungdahlii*. *Iran. J. Biotechnol.* **2006**, *4*, 45–53.
134. Methods for Increasing the Production of Ethanol from Microbial Fermentation. U.S. Patent Application No. 20030211585, 23 October 2007. Available online: <https://patents.google.com/patent/US20030211585A1/en> (accessed on 1 November 2020).
135. Acharya, B.; Dutta, A.; Basu, P. Ethanol production by syngas fermentation in a continuous stirred tank bioreactor using *Clostridium ljungdahlii*. *Biofuels* **2019**, *10*, 221–237. [CrossRef]
136. Ahmed, A.; Cateni, B.G.; Huhnke, R.L.; Lewis, R.S. Effects of biomass-generated producer gas constituents on cell growth, product distribution and hydrogenase activity of *Clostridium carboxidivorans* P7T. *Biomass Bioenergy* **2006**, *30*, 665–672. [CrossRef]
137. Ukpong, M.N.; Atiyeh, H.K.; De Lorme, M.J.M.; Liu, K.; Zhu, X.; Tanner, R.S.; Wilkins, M.R.; Stevenson, B.S. Physiological response of *Clostridium carboxidivorans* during conversion of synthesis gas to solvents in a gas-fed bioreactor. *Biotechnol. Bioeng.* **2012**, *109*, 2720–2728. [CrossRef]
138. Heffernan, J.K.; Valgepea, K.; de Souza Pinto Lemgruber, R.; Casini, I.; Plan, M.; Tappel, R.; Simpson, S.D.; Köpke, M.; Nielsen, L.K.; Marcellin, E. Enhancing CO<sub>2</sub>-Valorization Using *Clostridium autoethanogenum* for Sustainable Fuel and Chemicals Production. *Front. Bioeng. Biotechnol.* **2020**, *8*, 1–10. [CrossRef] [PubMed]
139. Sun, X.; Atiyeh, H.K.; Zhang, H.; Tanner, R.S.; Huhnke, R.L. Enhanced ethanol production from syngas by *Clostridium ragsdalei* in continuous stirred tank reactor using medium with poultry litter biochar. *Appl. Energy* **2019**, *236*, 1269–1279. [CrossRef]
140. Kundiyana, D.K.; Huhnke, R.L.; Wilkins, M.R. Syngas fermentation in a 100-L pilot scale fermentor: Design and process considerations. *J. Biosci. Bioeng.* **2010**, *109*, 492–498. [CrossRef]
141. Asimakopoulos, K.; Gavala, H.N.; Skiadas, I.V. Reactor systems for syngas fermentation processes: A review. *Chem. Eng. J.* **2018**, *348*, 732–744. [CrossRef]
142. Datar, R.P.; Shenkman, R.M.; Cateni, B.G.; Huhnke, R.L.; Lewis, R.S. Fermentation of biomass-generated producer gas to ethanol. *Biotechnol. Bioeng.* **2004**, *86*, 587–594. [CrossRef]

143. Rajagopalan, S.; Datar, R.P.; Lewis, R.S. Formation of ethanol from carbon monoxide via a new microbial catalyst. *Biomass Bioenergy* **2002**, *23*, 487–493. [CrossRef]
144. Devarapalli, M.; Atiyeh, H.K.; Phillips, J.R.; Lewis, R.S.; Huhnke, R.L. Ethanol production during semi-continuous syngas fermentation in a trickle bed reactor using *Clostridium ragsdalei*. *Bioresour. Technol.* **2016**, *209*, 56–65. [CrossRef]
145. Biological Production of Ethanol from Waste Gases with *Clostridium ljungdahlii*. U.S. Patent 6136577, 24 October 2000. Available online: <https://patents.google.com/patent/US6136577A/en> (accessed on 1 November 2020).
146. Riegler, P.; Bieringer, E.; Chrusciel, T.; Stärz, M.; Löwe, H.; Weuster-Botz, D. Continuous conversion of CO<sub>2</sub>/H<sub>2</sub> with *Clostridium aceticum* in biofilm reactors. *Bioresour. Technol.* **2019**, *291*, 121760. [CrossRef]
147. Yasin, M.; Jeong, Y.; Park, S.; Jeong, J.; Lee, E.Y.; Lovitt, R.W.; Kim, B.H.; Lee, J.; Chang, I.S. Microbial synthesis gas utilization and ways to resolve kinetic and mass-transfer limitations. *Bioresour. Technol.* **2015**, *177*, 361–374. [CrossRef] [PubMed]
148. Modular Membrane Supported Bioreactor for Conversion of Syngas Components to Liquid Products. U.S. Patent 8,828,692, 9 September 2014.
149. Datta, R.; Tsai, S.-P.; Basu, R.; Yoon, S.-H. Membrane Supported Bioreactor for Conversion of Syngas Components to Liquid Products 2009. U.S. Patent Application No. 20090017514, 15 January 2009.
150. Lee, P. Syngas fermentation to ethanol using innovative hollow fiber membrane. *Grad. Diss.* **2010**. [CrossRef]
151. Shen, Y.; Brown, R.; Wen, Z. Syngas fermentation of *Clostridium carboxidivorans* P7 in a hollow fiber membrane biofilm reactor: Evaluating the mass transfer coefficient and ethanol production performance. *Biochem. Eng. J.* **2014**, *85*, 21–29. [CrossRef]
152. Kantzow, C.; Mayer, A.; Weuster-Botz, D. Continuous gas fermentation by *Acetobacterium woodii* in a submerged membrane reactor with full cell retention. *J. Biotechnol.* **2015**, *212*, 11–18. [CrossRef]
153. Roy, S.; Bauer, T.; Al-Dahhan, M.; Lehner, P.; Turek, T. Monoliths as multiphase reactors: A review. *AIChE J.* **2004**, *50*, 2918–2938. [CrossRef]
154. Shen, Y.; Brown, R.; Wen, Z. Enhancing mass transfer and ethanol production in syngas fermentation of *Clostridium carboxidivorans* P7 through a monolithic biofilm reactor. *Appl. Energy* **2014**, *136*, 68–76. [CrossRef]
155. Devarapalli, M.; Lewis, R.S.; Atiyeh, H.K. Continuous ethanol production from synthesis gas by *Clostridium ragsdalei* in a trickle-bed reactor. *Fermentation* **2017**, *3*, 23. [CrossRef]
156. Daniell, J.; Köpke, M.; Simpson, S.D. Commercial biomass syngas fermentation. *Energies* **2012**, *5*, 5372–5417. [CrossRef]
157. Trevethick, S.R.; Bromley, J.C.; Simpson, S.D.; Khosla, V.; Lanza Tech New Zealand Ltd. Fermentation of Gaseous Substrates. U.S. Patent 8,178,330, 15 May 2012.
158. Li, X.; Cossey, B.J.; Trevethick, S.R.; LanzaTech New Zealand Ltd. Fermentation of Gaseous Substrates. U.S. Patent 9,617,509, 11 April 2017.
159. Fermentation of Gaseous Substrates. U.S. Patent 9617509B2. Available online: <https://patents.google.com/patent/US9617509B2/en> (accessed on 21 November 2020).
160. Aemetis Completes Operation of Cellulosic Ethanol demo Unit; 12 Mgy Plant for 2019-Green Car Congress. Available online: <https://www.greencarcongress.com/2018/03/20180309-aemetis.html> (accessed on 1 November 2020).
161. Anis, S.; Zainal, Z.A. Tar reduction in biomass producer gas via mechanical, catalytic and thermal methods: A review. *Renew. Sustain. Energy Rev.* **2011**, *15*, 2355–2377. [CrossRef]
162. Xu, D.; Lewis, R.S. Syngas fermentation to biofuels: Effects of ammonia impurity in raw syngas on hydrogenase activity. *Biomass Bioenergy* **2012**, *45*, 303–310. [CrossRef]
163. Ahmed, A.; Lewis, R.S. Fermentation of biomass-generated synthesis gas: Effects of nitric oxide. *Biotechnol. Bioeng.* **2007**, *97*, 1080–1086. [CrossRef]
164. Oswald, F.; Zwick, M.; Omar, O.; Hotz, E.N.; Neumann, A. Growth and product formation of *Clostridium ljungdahlii* in presence of cyanide. *Front. Microbiol.* **2018**, *9*, 1–12. [CrossRef]
165. Asadullah, M. Biomass gasification gas cleaning for downstream applications: A comparative critical review. *Renew. Sustain. Energy Rev.* **2014**, *40*, 118–132. [CrossRef]
166. Kumar, A.; Jones, D.D.; Hanna, M.A. Thermochemical biomass gasification: A review of the current status of the technology. *Energies* **2009**, *2*, 556–581. [CrossRef]

167. Huber, G.W.; Iborra, S.; Corma, A. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem. Rev.* **2006**, *106*, 4044–4098. [[CrossRef](#)]
168. Woolcock, P.J.; Brown, R.C. A review of cleaning technologies for biomass-derived syngas. *Biomass Bioenergy* **2013**, *52*, 54–84. [[CrossRef](#)]
169. Abdoulmoumine, N.; Adhikari, S.; Kulkarni, A.; Chattanathan, S. A review on biomass gasification syngas cleanup. *Appl. Energy* **2015**, *155*, 294–307. [[CrossRef](#)]
170. Zwart, R.W.R. Gas cleaning downstream biomass gasification. *Sent. Energy Res. Center Neth.* **2009**. [[CrossRef](#)]
171. Vasudevan, D.; Richter, H.; Angenent, L.T. Upgrading dilute ethanol from syngas fermentation to n-caproate with reactor microbiomes. *Bioresour. Technol.* **2014**, *151*, 378–382. [[CrossRef](#)]
172. Oswald, F.; Dörsam, S.; Veith, N.; Zwick, M.; Neumann, A.; Ochsenreither, K.; Syldatk, C. Sequential mixed cultures: From syngas to malic acid. *Front. Microbiol.* **2016**, *7*, 1–12. [[CrossRef](#)]
173. Lagoa-Costa, B.; Abubackar, H.N.; Fernández-Romasanta, M.; Kennes, C.; Veiga, M.C. Integrated bioconversion of syngas into bioethanol and biopolymers. *Bioresour. Technol.* **2017**, *239*, 244–249. [[CrossRef](#)]
174. Perez, J.M.; Richter, H.; Loftus, S.E.; Angenent, L.T. Biocatalytic reduction of short-chain carboxylic acids into their corresponding alcohols with syngas fermentation. *Biotechnol. Bioeng.* **2013**, *110*, 1066–1077. [[CrossRef](#)]
175. Isom, C.E.; Nanny, M.A.; Tanner, R.S. Improved conversion efficiencies for n-fatty acid reduction to primary alcohols by the solventogenic acetogen “*Clostridium ragsdalei*”. *J. Ind. Microbiol. Biotechnol.* **2015**, *42*, 29–38. [[CrossRef](#)]
176. Hwang, H.W.; Yoon, J.; Min, K.; Kim, M.S.; Kim, S.J.; Cho, D.H.; Susila, H.; Na, J.G.; Oh, M.K.; Kim, Y.H. Two-stage bioconversion of carbon monoxide to biopolymers via formate as an intermediate. *Chem. Eng. J.* **2020**, *389*, 124394. [[CrossRef](#)]
177. Diender, M.; Stams, A.J.M.; Sousa, D.Z. Pathways and bioenergetics of anaerobic carbon monoxide fermentation. *Front. Microbiol.* **2015**, *6*, 1–18. [[CrossRef](#)]
178. Choi, D.; Chipman, D.C.; Bents, S.C.; Brown, R.C. A techno-economic analysis of polyhydroxyalkanoate and hydrogen production from syngas fermentation of gasified biomass. *Appl. Biochem. Biotechnol.* **2010**, *160*, 1032–1046. [[CrossRef](#)]
179. Kerby, R.L.; Hong, S.S.; Ensign, S.A.; Coppoc, L.J.; Ludden, P.W.; Roberts, G.P. Genetic and physiological characterization of the *Rhodospirillum rubrum* carbon monoxide dehydrogenase system. *J. Bacteriol.* **1992**, *174*, 5284–5294. [[CrossRef](#)]
180. Do, Y.S.; Smeenk, J.; Broer, K.M.; Kisting, C.J.; Brown, R.; Heindel, T.J.; Bobik, T.A.; DiSpirito, A.A. Growth of *Rhodospirillum rubrum* on synthesis gas: Conversion of CO to H<sub>2</sub> and poly-β-hydroxyalkanoate. *Biotechnol. Bioeng.* **2007**, *97*, 279–286. [[CrossRef](#)]
181. Najafpour, G.D.; Younesi, H. Bioconversion of synthesis gas to hydrogen using a light-dependent photosynthetic bacterium, *Rhodospirillum rubrum*. *World J. Microbiol. Biotechnol.* **2007**, *23*, 275–284. [[CrossRef](#)]
182. Najafpour, G.; Younesi, H.; Mohamed, A.R. Effect of organic substrate on hydrogen production from synthesis gas using *Rhodospirillum rubrum*, in batch culture. *Biochem. Eng. J.* **2004**, *21*, 123–130. [[CrossRef](#)]
183. Revelles, O.; Tarazona, N.; García, J.L.; Prieto, M.A. Carbon roadmap from syngas to polyhydroxyalkanoates in *Rhodospirillum rubrum*. *Environ. Microbiol.* **2016**, *18*, 708–720. [[CrossRef](#)] [[PubMed](#)]
184. Brandl, H.; Knee, E.J.; Fuller, R.C.; Gross, R.A.; Lenz, R.W. Ability of the phototrophic bacterium *Rhodospirillum rubrum* to produce various poly (β-hydroxyalkanoates): Potential sources for biodegradable polyesters. *Int. J. Biol. Macromol.* **1989**, *11*, 49–55. [[CrossRef](#)]
185. Karmann, S.; Panke, S.; Zinn, M. Fed-batch cultivations of *rhodospirillum rubrum* under multiple nutrient-limited growth conditions on syngas as a novel option to produce poly(3-hydroxybutyrate) (PHB). *Front. Bioeng. Biotechnol.* **2019**, *7*, 1–11. [[CrossRef](#)] [[PubMed](#)]
186. Najafpour, G.; Younesi, H.; Mohamed, A.R. Continuous hydrogen production via fermentation of synthesis gas. *Petrol Coal. Pet. Coal* **2013**, *45*, 154–158.
187. Younesi, H.; Najafpour, G.; Ku Ismail, K.S.; Mohamed, A.R.; Kamaruddin, A.H. Biohydrogen production in a continuous stirred tank bioreactor from synthesis gas by anaerobic photosynthetic bacterium: *Rhodospirillum rubrum*. *Bioresour. Technol.* **2008**, *99*, 2612–2619. [[CrossRef](#)]
188. Sugimoto, T.; Tsuge, T.; Tanaka, K.; Ishizaki, A. Control of acetic acid concentration by pH-Stat continuous substrate feeding in heterotrophic culture phase of two-stage cultivation of *Alcaligenes eutrophus* for

- production of P(3HB) from CO<sub>2</sub>, H<sub>2</sub>, and O<sub>2</sub> under non-explosive conditions. *Biotechnol. Bioeng.* **1999**, *62*, 625–631. [[CrossRef](#)]
189. Ishizaki, A.; Tanaka, K.; Taga, N. Microbial production of poly-D-3-hydroxybutyrate from CO<sub>2</sub>. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 6–12.
190. Ishizaki, A.; Taga, N.; Takeshita, T.; Sugimoto, T.; Tsuge, T.; Tanaka, K. Microbial Production of Biodegradable Plastics from Carbon Dioxide and Agricultural Waste Material. *ACS Symp. Ser.* **1997**, *666*, 294–306.
191. Taga, N.; Tanaka, K.; Ishizaki, A. Effects of rheological change by addition of carboxymethylcellulose in culture media of an air-lift fermentor on poly-D-3-hydroxybutyric acid productivity in autotrophic culture of hydrogen-oxidizing bacterium, *alcaligenes eutrophus*. *Biotechnol. Bioeng.* **1997**, *53*, 529–533. [[CrossRef](#)]
192. Volova, T.G.; Kalacheva, G.S.; Altukhova, O.V. Autotrophic synthesis of polyhydroxyalkanoates by the bacteria *Ralstonia eutropha* in the presence of carbon monoxide. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 675–678.
193. Heinrich, D.; Raberg, M.; Steinbüchel, A. Studies on the aerobic utilization of synthesis gas (syngas) by wild type and recombinant strains of *Ralstonia eutropha* H16. *Microb. Biotechnol.* **2018**, *11*, 647–656. [[CrossRef](#)] [[PubMed](#)]
194. Fuhrmann, S.; Ferner, M.; Jeffke, T.; Henne, A.; Gottschalk, G.; Meyer, O. Complete nucleotide sequence of the circular megaplasmid pHCG3 of *Oligotropha carboxidovorans*: Function in the chemolithoautotrophic utilization of CO, H<sub>2</sub> and CO<sub>2</sub>. *Gene* **2003**, *322*, 67–75. [[CrossRef](#)]
195. Paul, D.; Bridges, S.M.; Burgess, S.C.; Dandass, Y.S.; Lawrence, M.L. Complete genome and comparative analysis of the chemolithoautotrophic bacterium *Oligotropha carboxidovorans* OM5. *BMC Genom.* **2010**, *11*, 511. [[CrossRef](#)]
196. "SYNPOL.". Available online: <http://www.synpol.org/> (accessed on 21 November 2020).

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).